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Review

The plexin-B family and its role in cancer progression

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Summary. Plexins are transmembrane protein receptors for semaphorin molecules. These molecules are involved in numerous cellular activities related to cell proliferation, adhesion along with the basement membrane, cellular motility and invasive capability. All nine members of Plexins identified in vertebrates have been grouped into subclasses, termed Plexin-A, Plexin -B, Plexin-C and Plexin-D. Plexin-B consists of three members, namely Plexin-B1, Plexin-B2 and Plexin-B3. Plexin-B1 functionally interacts with Sema4A (Yukawa et al., 2010) and can also form heterodimer with Plexin-B2 for Sema4A binding (Nkyimbeng-Takwi and Chapoval, 2011). Plexin-B2 binds with Sema4C. Plexin-B3 mediates interaction with both Sema4G and Sema5A. Some semaphorines exist in a membrane-bound form only, whereas other family members can be found in tissues/fluids in both secreted and membrane-bound forms. This ligand-receptor interaction between sema4D and Plexin-B1 indicated in different signaling pathways results in many intriguing and interesting findings, highlighting its importance in both physiology and pathology. Apart from bidirectional signaling among these molecules, the involvement of Plexin-B1 in the processes described here directly involves a bidirectional singaling between Sema4Dand Plexin-B1. Being a high affinity receptor for both Sema4A and Sema4D, the role played by Plexin-B1 in cancer progression, metastasis and angiogenesis is still an area requiring further research. Activation of Sema4D mediated downstream effectors is largely influenced by cross talk of Plexin-B1 with other molecules, such as Her-2 and Met. In this review, all findings regarding Plexin-B1 upstream and downstream regulation and its putative involvement in relation to the ultimate fate of cancer cells are discussed.

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Introduction

Plexins are transmembrane proteins acting as receptors for the semaphroin family (Kumanogoh and Kikutani, 2010). Plexins were initially identified in *Xenopus Laevis* and were named due to the observed staining of the plexiform layer of the optic tectum tissue (Takagi et al., 1987; Ohta et al., 1995). Semaphorins require Plexins, either alone or together with neuropilin proteins, to mediate their effects in different organs. These molecules were initially found to be responsible for the directional growth of axons, acting as guidance cues and were thus termed as semaphorins (Semaphorin Nomenclature Committee, 1999). Members of the class 4 to 7 semaphorins (membrane bound proteins), along with one member of class 3 (sema3A), bind directly to their specific Plexins and activate Plexin-mediated signal transduction. Sema3A, sema3B, sema3C, and sema3F mediate their effect by interacting with the neuropilin/Plexin complex. Certain members of class 4 such as Sema4D may exist in soluble format which binds to Plexin-B1 with high affinity and Plexin-B2 with low affinity (Tamagnone et al., 1999; Masuda et al., 2004). Apart from their conventional receptors (Plexins) some semaphorins do interact with other molecules as their cognate receptors. For example, integrin for sema7A (Suzuki et al., 2007); CD72 for sema4D (Kumanogoh et al., 2000) and TIM-2 for sema4A (Kumanogoh et al., 2002). To date four families of Plexins have been identified in both human and mouse genomes, namely Plexin-A, Plexin-B, Plexin-C and Plexin-D. Plexin-A contains four members (Plexin-A1, -A2, -A3 and -A4); Plexin-B contains three members (Plexin-B1, -B2 and -B3); Plexin-C contains one

member (Plexin-C) and Plexin-D contains one member (Plexin-D) (Kruger et al., 2005). Details of about these structure can be seen in a recent review (Nkyimbeng-Takwi and Chapoval, 2011). In addition to acting as axonal guidance cues in the nervous system, the Plexin family have also been shown to be involved in several other physiological processes, including vascular growth (Gu et al., 2003, 2005; Serini et al., 2003), follicle maturation (Regev et al., 2007), heart morphogenesis (Gitler et al., 2004) and immunity (Kikutani et al., 2007). Recently, a significant influence of these molecules in regulating recovery processes from cardiovascular, immune, skeletal and nervous system injury has also been observed (Perala et al., 2012).

Plexins constitute a ligand binding domain (Sema) along with two or three MET related sequences in their extracellular portion which links to the intracellular region via a single transmembrane helix structure (Tamagnone et al., 1999) as shown in Figure 1. The extracellular region also contains several glycine and proline rich immunogobulin domains providing a binding site for different transcription factors and integrins (Bork et al., 1999). The intracellular portions of Plexins are highly conserved, lacking kinase activity. However, the intracellular portion contains both Ras as well as RhoGTPase binding regions. Plexins are the only class of transmembrane proteins which interact with a range of GTPases via a Ras GTPase activating domain and a RhoGTPase binding domain (Negishi et al., 2005; Panizzi et al., 2007; Bell et al., 2011). The intracellular portion of Plexins contains a Sex-Plex domain which is conserved among different Plexins and across species (Oinuma et al., 2004). This domain also exhibits partial similarity with the GTPase activating protein (GAP)

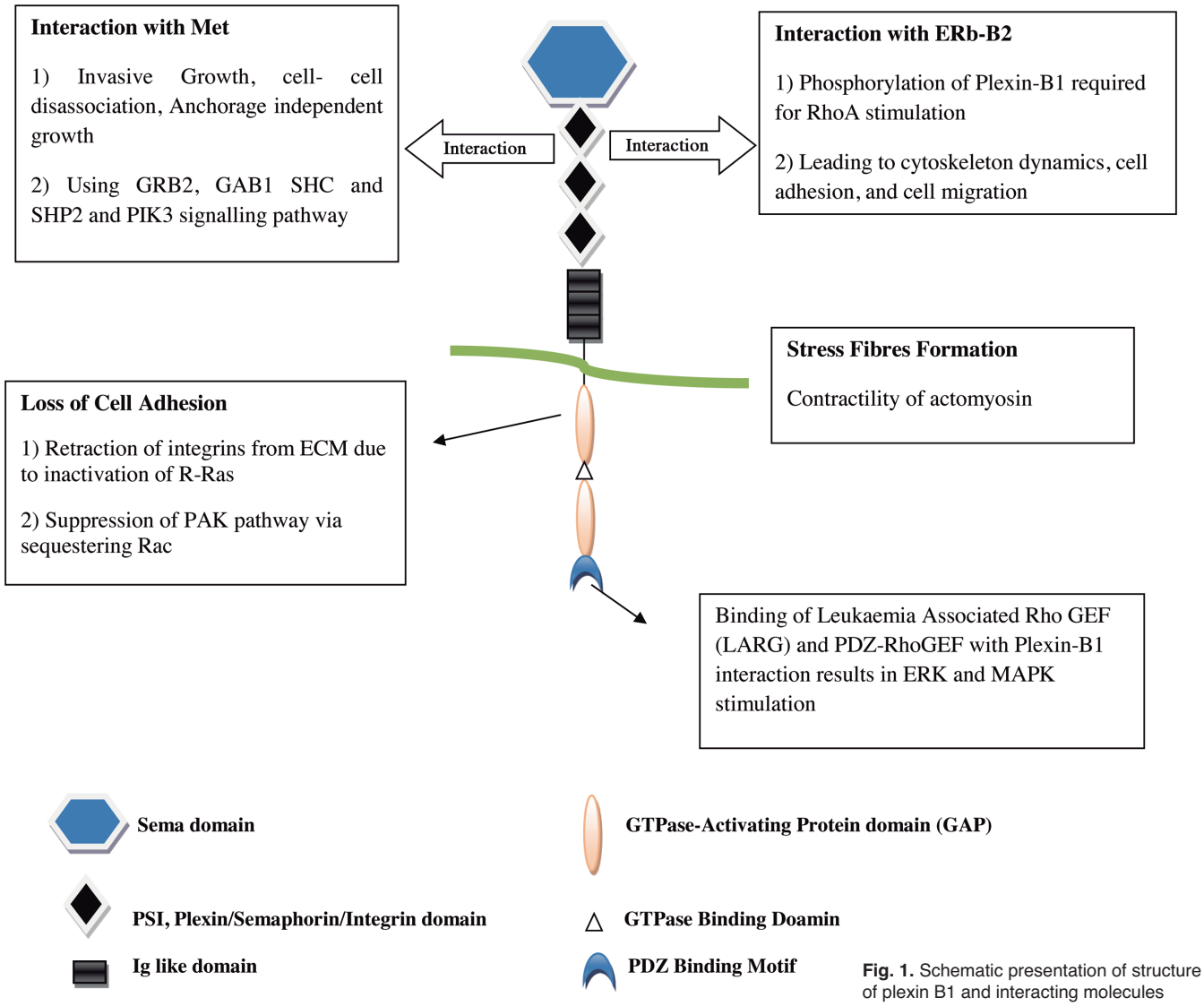

Fig. 1. Schematic presentation of structure of plexin B1 and interacting molecules

domain of p120 RasGAP (Oinuma et al., 2006).

All three Plexin-B members contain a proprotein convertase cleavage site in their extracellular region (Artigiani et al., 2003). Once cleaved, the extracellular portion of Plexin-B1 acts as an inhibitory moiety for Plexin-B1 mediated downstream regulation. It contains a consensus sequence of tetrabasic amino acid ($R_{1302}XXR$) which is a target site for subtilisin like proprotein convertases (PC) cleavage (Tamagnone et al., 1999). After cleavage, the single chain peptide is cleaved into non disulfide linked hetrodimeric fragments. To date, the fate of the cleaved extracellular portion of Plexin-B1, either outside the cell or as a membrane bound entity, has not yet been explored. However, the cleaved form of Plexin-B1 has been found in a variety of cell lines (liver, pancreas, gastric and colon) and human tissues (kidney, lung, breast, pancreas and colon). Interestingly, site specific cleavage of the Plexin-B1 precursor is regulated only on the cell membrane surface and duly effects Plexin-B1 bindng affinity with sema4D (Artigiani et al., 2003).

Genetic and molecular features

Structure and Function of Plexin-B1

Plexin-B1 was identified on the basis of sequence similarities with the hepatocyte growth receptor family (HGF) and its predominant expression in human brain and kidney tissues (Maestrini et al., 1996). *Plexin-B1*, also termed *SEP*, is localizsed on chromosome 3p21, whereas *Plexin-B2* is found on 22q13 and *Plexin-B3* on Xq28 (Tamagnone et al., 1999). Membrane bound Plexin-B1 comprises 2135 amino acids, encoded from 38 exons, and is responsible for mediating sema4D axon guidance cue signals through activated Rac (Vikis et al., 2000). Plexin-B1 and the HGF receptor (c-Met) share a conserved extracellular portion of 500 amino acids (sema domain) and a cysteine motif of 80 amino acids (Met related sequence; MRS) (Tamagnone et al., 1999; Kolodkin et al., 1993). A synergistic correlation in the expression pattern of Plexin-B1 and c-Met have been observed (Giordano et al., 2002). Structural homology of the extracellular domain of Plexin-B1 with hepatocyte growth factor receptor (c-Met) also suggests its involvement in invasive cell proliferation, loss of adhesion, migration and localization at different sites. The intracellular region of Plexin contains Ras GTPase activating protein (GAP) and RhoGTPase binding portion (RBD) domains (Rohm et al., 2000). Both these domains are responsible for downstream mediation of the semaphorin induced repulsive chemotactic response (He et al., 2009). Structurally, Plexins contain a signal peptide domain (SP), semaphorin domain (sema), three PSI domains (Plexin, semaphorin, and integrin), six IPT domains (immunoglobulin like, Plexins, transcription factors), a cleavage site (CS) and a transmembrane region (TM). Two highly conserved regions (C1 and C2) are present in the cytoplasmic portion of Plexin B1 (Fig. 1).

Processing of Plexin-B1 leads to the formation of two heterodimeric via non disulfide linked extracelleular units. The alpha subunit consists mostly of extracellular region while the beta subunit constitutes a relatively small extracellular portion along with trans membrane and cytoplasmic regions. No disulphide bond formation exists among alpha and beta subunits and leads to speculation that this interaction may dissociate under certain conditions (Artigiani et al., 2003).

Interestingly, Sema4D-mediated Plexin-B1 receptor activation requires dimerization of the N-terminal domains of another Plexin-B1 molecule. This dimerization results from bivalent semaphorin binding, as observed by crystallography and multiple angle light scattering experimentation (Janssen et al., 2010). Intercalated dimerization of Plexins and bivalent activation of semaphroins has also been identified in other Plexin and semaphorin family members, highlighting a common mode of activation for apposing cells.

The location of genetic mutations on Plexin-B1 regulates cell response in relation to cancer cell adhesion, invasion and motility. Mutations confined to the cytoplasmic portion of Plexin-B1 primarily affect cell attachment to various substrates, whereas mutation encountered in the extracellular region may affect cellular motility and invasiveness, as both key players (c-Met and HER-2) interact with the extracellular portion (Giordano et al., 2002; Swiercz et al., 2004). Being a trans-membrane protein, Plexin-B1 receptor has a short cytoplasmic domain responsible for interacting with a wide range of molecules. Lacking any intrinsic kinase activity, this portion mediates cell signalling through interactions with various associated GTPases. Mutation at the C-terminus of Plexin-B1 leads to failure of binding with PDZ/RhoGEF, leading to impaired cytoskeleton rearrangement (Lin et al., 2007). Somatic missense mutation (L1815P) on Plexin-B1 impairs its binding affinity with Rac. This mutation prevents binding of Plexin-B1 with Rac-GTP which subsequently results in the phosphrylation of p21-activated kinase (Pak1), leading to cell spreading and lamellipodia extension (Zhou et al., 2012a,b). In prostate cancer cells, somatic mutations occuring at the intracellular portion of Plexin-B1 have been associated with bone and lymph node metastasis (Wong et al., 2007). Hence, mutations on Plexin-B1 also impart a significant role in impairing its effects on downstream effector molecules like RhoA.

Structure and Function of Plexin-B2

A gene for *Plexin-B2* is localized on chromosome 22, comprising 37 coding exons. This transmembrane protein is composed of 1838 amino acids (Nagase et al., 1997). All members of the Plexin B family contain an intracellular domain with a PDZ motif (post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), and zonula occludens-1 protein (zo-1)), which can relay extracellular signals to intracellular motifs. This motif interacts with the PDZ domain of LARG (Leukemia Associated Rho GEF) and Rho-GEF

molecules resulting in the stimulation of endogenous RhoA and reorganization of the cytoskeleton (Perrot et al., 2002). Plexin-B2 interacts with both sema4D as well as sema4C molecules (Masuda et al., 2004) and is responsible for intracellular signalling of hepatocyte growth factor-mediated cellular motility. Expression of Plexin-B2 has been observed in specialized endothelial cells in endocrine glands, bronchial epithelial, islets of Langerhans and adrenal glands (Zielonka et al., 2010). Plexin-B2 has an impact on cell adhesion by mediating homophilic interaction under the influence of calcium ions (Ohta et al., 1995). A significant impact of Plexin-B2 in skin wound healing has also been observed via ligand sema4D stimulated ERK pathway activation (Witherden et al., 2012). In another study, the lack of Plexin-B2 led to abnormal cortical layer formation and impaired migratory ability of subtypes of cortical neurons in mice, while loss of Plexin-B1 did not mediate any developmental abnormalities (Hirschberg et al. 2010). Both these studies highlight an interesting point of receptor overlap for sema4Dmediated effects on cortical neurons as well as non neuronal cells (Hirschberg et al., 2010; Zielonka et al., 2010). Since both Plexin-B1 and Plexin-B2 are regulated by sema4D ligand, a non-redundant role of both these Plexin molecules in different non neural organs is a proposed area that requires further research. Association of sema4D along with Plexin-B2 and Plexin-B1 has also been observed in neural cells. Apart from restricting growth cues, sema4D is also responsible for increased proliferation of dorsal root ganglion. These cells grow under the conditional presence of both nerve growth factor and tropomysoin receptor kinase A (TrKA) receptor (Masuda et al., 2004). Apart from sema4D, higher expression of sema4A in early embryonic development, brain, spleen, kidney and breast and its interaction with Plexin-B1 has also been reported (Kumanogoh et al., 2002; Yukawa et al., 2010). Both sema4A and sema4D mediate the same growth cone collapse in hippocampal neurons. This fact highlights a diversified interacting range of semaphorins with Plexin-B1 in relation to different tissues and diseases as well.

Structure and function of Plexin-B3

Plexin-B3 is localized on chromosome Xq28, comprising 35 coding exons. It is a transmembrane protein consisting of 1932 amino acids. Plexin-B3 is a receptor for sema5A which has been found to be responsible for the intracellular signalling of hepatocyte growth factor-mediated cellular motility (Artigiani et al., 2004). Interaction of Plexin-B3, A2 and B1 with microtubule plus end-binding proteins EB1, EB2 and EB3 have been found to be responsible for neurite growth (Laht et al., 2012). Ca^{2+}/Mg^{2+} dependent homophilic interactions of Plexin-B3 have also been found to be responsible for cell aggregation. Plexin-B3 positively affects neuronal morphology independently from semaphorin signalling (Hartwig et al., 2005). Surprisingly, no adverse effects either on brain or spinal cord, following the loss of Plexin-B3 in mice has been reported in the literature (Worzfeld et al., 2009). This lack of effect following Plexin-B3 loss has been attributed to the putative involvement of several other functionally redundant semaphorin/Plexins, Eph/ephrins, PDGF/PDGFR, FGF-2/FGFR interactions.

Plexin-B1 is the most extensively studied receptor among this family. Altered expression of this molecule has been indicated in different types of cancers as shown in table 1. In the present review the significance of this molecule in relation to physiological and pathological states has been addressed.

Interacting molecules and signaling mechanism of Sema4D and Plexin-B1

Upstream regulators for Sema4D and Plexin-B1

CD38 can act upstream of Plexin-B1 (Deaglio et al., 2005). Stringent regulation of CD38 on B-cell

Table 1. Expression of Plexin-B1 in different cancers.

Type of cancer	Clinical findings	Reference
Prostate	Higher protein intensity observed in neoplastic in comparison to non neoplastioc tissues, Similar trend observed in mRNA levels when compared among primary cancer and non-neoplastic tissues	Wong et al., 2007
Renal Carcinoma	Reduced expression of Plexins-B1 in renal carcinoma cells (169 out of 209) in comparison to normal renal cells using immunohistochemistry	Gomez Roman et al., 2008
Breast Cancer	Loss of Plexin-B1 was correlated with poor disease free survival and overall survival correlated expression in Stem cell like ER positive cancer and tumour proliferation	Rody et al., 2007, 2008
Ovarian Cancer	Co-expression of both plex-B1 and Met was associated with worse grade and higher incidence of lymph node metastases	Valente et al., 2009
	Immunoreactivity showed gradual increase from stage I to stage IV but lack any statistical significance. However, Plexin-B1 directly involved in lymphatic metastasis in a cohort of 120 tissue	Ye et al., 2010
Skin Cancer	Higher expression of Plexin-B1 observed in benign and primary melanoma as compared to metastatic melanomas showing reduced/loss of signals. Stimulated B-Raf/MKK cascade also lead to suppression of Plexin-B1 in WM239A	Stevens et al., 2010; Argast et al ., 2009
Cervical Cancer	Over expression of Plexin-B1 in cervical tumours (n=21) as compared to normal cervical tissues (n=40)	Qiang et al., 2011

ontogenesis is observed and maximal expression of CD38 is seen in bone marrow precursors. CD38 expression is higher during early B-cell precursor development and plasma cell differientation. Reduced levels of CD38 have been observed in normal B cells which reappear in terminally differentiated plasma cells (Malavasi et al., 1994). In B-cell chronic lymphocytic leukaemia (CLL), CD38 (receptor) and CD31 (ligand) interaction upregulates Sema4D, acting as a growth promoter and cell survival factor (Kumanogoh and Kikutani, 2001, 2003). Expression of both Sema4D and Plexin-B1 led to an increase in life span of B cells in CLL patients (Deaglio et al., 2006). Synergistic activation of both CD38 and ZAP70 (T-cell marker) lead to poor survival and disease progression in CLL (Deaglio et al., 2007). The effect of CD38 on phosphorylation of ZAP70 in B cell lineage is an area that requires further research. Hence, CD38 cross talks with Sema4D, Plexin-B1 (upregulation) and CD72 (down regulation), orchestrates cells proliferation, survival and differentiation. Similarly, hypoxia induced factor (HIF-1) influences Sema4D expression. Upstream localized hypoxia response elements (HRE) increase Sema4D expression resulting in the promotion of angiogenesis, and increased tumour growth and invasiveness through interaction of Sema4D with Plexin-B1 receptor (Siebold et al., 2005; Sun et al., 2008). Hence, it is worthwhile exploring the influence of hypoxic conditions on the expression profiles of both molecules in different organs. Increased expression of Sema4D, its high affinity receptor (Plexin-B1), has been observed in proliferating B-cells accompanied by concomitant loss of CD72 (Kumanogoh et al., 2000; Deaglio et al., 2005). These elements provide a novel cue in CLL where CD38/CD31 interactions can influence Sema4D expression and Plexin-B1 activation through a ZAP70 dependent pathway.

Plexin-B1 interactions with molecules

Plexin-B1 receptor interaction with cMet, Rho GTPases Rac1, RhoA, Erb 2 and F-actin has been extensively reported in the literature. Depending upon the location, intensity and interacting molecule these interactions mediate variable and sometimes quite contrasting results. Extracellular domains of all Semaphorins, Plexins and hepatocyte growth factor receptors share structural homology (Sema and MRS; Met related sequence regions) (Winberg et al., 1998; Tamagnone et al., 1999). Binding of Sema4D with Plexin-B1 stimulates Met kinase activity inducing tyrosine phosphorylation of both Plexin-B1 and Met receptors (Giordano et al., 2002; Chedotal et al., 2005). Interaction between Plexin-B1 and Met are essential for epithelial cell anchorage, independent growth and invasiveness. Other studies have shown the interaction of two members, M-Ras and R-Ras, with the GAP region of Plexin-B1 leads to suppression of ras mediated cellular function (Oinuma et al., 2004; Saito et al., 2009). In neural and melanoma cells, these interactions resulted in inactivation of integrin ,1, restricting cellular motility and reduced outgrowth (Oinuma et al., 2006; Argast et al., 2009). Interaction of Plexin-B1 with RhoGTPase members in their active forms is required for mobilizing the actin cytoskeleton (Negishi and Kato, 2002). ErBb2 mediated phosphrylation of Plexin-B1 activates downstream activators Rho A and Rho C, responsible for breast cancer cells invasiveness (Worzfeld et al., 2012). Hence, interaction of Plexin-B1 with Met extracellularly and with Rho and Ras intracellularly produces quite contrasting findings. These variable features may depend on the type of organ involved, and the localization and expression of recptors in the vicinity, as well as the interactions stimulated.

Interaction with GTPases

Plexin-B1 specifically interacts with Rac via its cytosolic Cdc42/Rac interactive binding (CRIB) motif encompassing 180 residues. The formation of a complex between Rac1 and the cytoplasmic domain of Plexin-B1 is one of the first documented cases of a direct interaction between a small guanosine 5'-triphosphatase (GTPase) and a transmemberane receptor Plexin (Bouguet-Bonnet and Buck, 2008). Upon the binding with ligand (Sema4D), Plexin-B1 also interacts with activated Rac via its cytosolic domain (Vikis et al., 2000). Active Rac does compete with Plexin-B1 and p21 activated kinase for its downstream effects. The interaction with Rac can reciprocally increase the binding affinity of Plexin-B1 with Sema4D which results in inhibition of Rac mediated PAK activation (Vikis et al., 2002). Activated PAK regulates a formation of filopodia and membrane ruffles (Frost et al., 1998), whilst inactivated PAK results in growth cones collapse as observed in Drosophila (Hu et al., 2001). Binding affinity of Rho on Plexin-B1 (via dbl homology domain) had been observed (Driessens et al., 2001). Interaction of RhoA using PDZ-RhoGEF and LARG (Leukemia-associated Rho guanine nucleotide exchange factor) with Plexin-B1 has also been established. Interestingly, the effect of Plexin-B1 on Rho mediated actin cytoskeletal contraction had been observed in all these reports (Driessens et al., 2001; Swiercz et al., 2002). PDZ-RhoGEF and LARG molecules are members of the Rho-GEF family and retain RGS (regulator of G protein Signaling) and Dbl homology domains. In mammalian cells, PDZ (PSD-95/SAP90-Discs-larg-ZO-1) domain recognition motif at the C terminus of Plexin-B family members specifically interacts with the PDZ domain of PDZ-RhoGEF. Interestingly, the binding affinity of Plexin-B1 with PDZ-RhoGEF/LARG is not influenced by the presence of Sema4D presence but this interaction increases recruitment of Plexin-B1 on cell membranes (Swiercz et al., 2002). LARG is a RhoA-specific guanine nucleotide exchange factor (GEF), and the PSD-95/Disc-large/ZO-1 homology domain of LARG interacts with Plexin-B1 for extracellular mediated

stimulation of RhoA (Liu et al., 2008).

The cytoplasmic Rho binding domain (RBD) of Plexin-B1 mapped has a binding interface for several molecules, including Rac1, Rnd1, and RhoD. These molecules are responsible for inducing several different signalling pathways within the cell (Hota and Buck, 2009). Interestingly, increased axonal cell proliferation and growth cone migration are largely influenced by a Rac dependent pathway (Lin and Greenberg, 2000). However, growth cone collapse via this Rac dependent pathway has also been reported in several published reports (Jin and Strittmatter, 1997; Kuhn et al., 1999; Vastrik et al., 1999), which is regulated by Plexin mediated Rho activation (Driessens et al., 2001). Furthermore, inactivation of R-Ras (exchange from GTP to GDP form) binding with Plexin-B1 (GAP domain) leads to the detachment of the cell from the extracellular matrix (ECM).

Apart from Plexin-B1 expression in the majority of organs (Hall et al., 1996; Furuyama et al., 1996), CD72 is also another low affinity receptor for Sema4D found in lymphocytes (Shi et al., 2000). Sema4D stimulation induced tyrosine dephosphorylation of CD72 and its dissociation from SHP-1. Inactivation of CD72 is important for B cell aggregation and growth (Wu et al., 1998; Ishida et al., 2003).

Bidirectional signalling

The role played by Plexins in bidirectional signalling from either out to inside the cell or from inside to outside the cell environment is still an evolving area requiring further research. Earlier reports have shown the existence of this dual channel molecular cross talk in relation to embryonic heart formation and B lymphotcyte differentiation (Billard et al., 2000; Comoglio et al., 2004). Forward signalling from Sema6D to Plexin-A1 stimulation is required for heart ventricular expansion, while reverse signalling using Plexin-A1 towards Sema6D is necessary for trabecular formation of chicken embryonic heart. In the forward signalling pathway, Plexin interaction via Her-2, off track tyrosine kinases (OTK), Met, VEGFR2 and their intercalated expressions do largely influence cellular motility. In reverse signalling, members of the vasodilator-stimulated phosphoprotein (Vasp) family are immuno precipitated with Sema6D. The cytoplasmic portion of Sema6D, which interacts with Abl kinases, is conserved within the family (Toyofuku et al., 2004). Hence, the Plexin-B1 ligand (Sema4D) may not be interacting with the same group of kinases. However, immuno precipitation of Sema4D along with protein tyrosine phosphatases (CD45) had also been observed. It had been shown that intracellular serine kinase showed stimulation of T-cell activation (Elhabazi et al., 2003). In addition, binding of calmodulin to the membrane-proximal cytoplasm domain can prevent the ADMA17-dependent cleavage of Sema4D (Mou et al., 2013). However, Sema4D mediated responses in relation to epithelial cells is an area that requires further research.

Role of plexin-B1 in different physiological systems

Effect of Plexin-B1 on the nervous system

Both Semaphorin and Plexin family proteins are inevitably involved in neuronal guidance, maturation and regulation of motility. Differenitial expressions of these proteins are also seen in different diseases related to the nervous system. Genetic aberrations in Sema5A and Sema3E are observed in Cri-du chat and CHARGE syndrome respectively (Lalani et al., 2004; Clarimon et al., 2006). Similarly, altered expression of Plexin-A2 and Plexin-B3 are associated with schizophrenia and impaired verbal performance (Wray et al., 2007; Rujescu et al., 2007). As safe guards of neuronal outgrowth, Plexins and Semaphorins are not highly expressed in mature neural networks. This is under strict control, even following injury, where class 3 and 4 Semaphorins are up- regulated in myelinated cells mediated via oligodendrocytes. These proteins are responsible for preventing excessive axon sprouting and migration (Moreau-Fauvarque et al., 2003; Shifman and Selzer, 2006). Plexin B members influence neurite outgrowth, axonal growth cone collapse and arborisation. Although loss of Plexin-B2 leads to abnormal development in the nervous system, deletion of either Plexin-B1 or Plexin-B3 do not result in phenotypic change in the nervous system of mice (Deng et al., 2007; Worzfeld et al., 2009). This suggests not only a compensatory role for both molecules (Plexin-B1 and Plexin-B2) but also a tissue specific receptor ligand interaction with Sema5A with Plexin-B3 as well (Hartwig et al., 2005). However, in another study, the importance of Plexin-B1 in gonadotropin releasing hormone (GnRH-1) secreting neuron motility was also reported. In the early embryonic phase, neurons specific for GnRH-1 were originally localized in the nasal placode. During embryonic development, these cells migrate to the hypothalamus under the stringent control of Semaphorins and other molecules. Motility of these cells is also mediated through the coupling of Plexin-B1 with the Met receptor tyrosine kinase upon Sema4D stimulation. Impaired expression of Plexin-B1 results in a substantial decrease of neuronal cell (responsible for GnRH-1 secretion) localization in adult brain leading to idiopathic hypogonadotropic hypogonadisms (IHH) (Giacobini et al., 2008).

Both Sema4D and Plexin-B1 molecules are also involved in the maturation of dendrites and neuronal spine maturation. Neuronal spine maturation following Sema4D exposure is believed to be mediated via the RhoA/ROCK signalling pathway, as treating the cells with ROCK inhibitor (Y-27632) restricts any elevation in spine density (Lin et al., 2007). After Sema4D stimulation, interaction of Plexin-B1 with PDZ-RhoGEFs via its C-terminal is important for induction of changes in dendritic spine. Although association of both

these molecules has been mentioned in several independent studies (Hirotani et al., 2002; Perrot et al., 2002; Swiercz et al., 2002) its role in relation to hippocampal neurons and RhoA/ROCK pathway has only recently been established (Lin et al., 2007). It has been observed that a Plexin-B1 mutant (lacking C-terminus region) was unable to interact with PDZ-RhoGEFs, leading to the blockage of Sema4D-induced dendritic spine density increase. In contrast to earlier studies, a decrease in fibroblast cell adhesiveness, mediated by Plexin-B1 activation was also found to be independent of the RhoA/ROCK pathway in both Cos-7 and 3T3 cells. Neither deletion of the PDZ domain binding motif from Plexin B1 nor treatment with Y-27632 (inhibitor for Rho dependent kinase) induced any substantial effect in cellular collapse. However, association of integrin and focal adhesion kinase do mediate cell retraction. The exogenous exposure of Sema4D to breast (SKBR3) and neuroblastoma (NB100) cell lines resulted in disassembly of focal complexes and relocation of integrin from the membrane to the cytoplasm (Barberis et al., 2004). The effect of both receptor and ligand interactions over subsequent stages of neurite development, differentiation and maturation have been explored and have shown the involvement of Plexin-B1 activated receptor tyrosine kinase, but no involvement of ERK pathway influx (Vodrazka et al., 2009).

Microglia are mononuclear phagocytes present in the brain and are active respondents of injury and infection. However, their persistent neuroinflammatory stimulation is also quite harmful to the neurons (Streit et al., 1999). The role of Sema4D and Plexin-B1 signalling in the restricted cellular motility of activated microglia has been observed (Toguchi et al., 2009). Thus, Sema4D and Plexin-B1 mediated pathways act as gatekeepers for repressing pro- inflammatory responses inside the brain.

In vitro assay results have demonstrated a strong binding affinity for mouse Plexin-A1 and Plexin-B1 molecules. Mouse Plexin-B1 amino acid sequence is 86% identical to human Plexin-B1 while its cytoplasmic region is 97% identical to human Plexin-B1 (Usui et al., 2003).

Impact of Plexin-B1 in non- neural organs

The role of Plexin-B1 has been explored in mouse embryonic tissue using non-radioactive *in situ* hybridisation. Plexin-B1 has been found to be expressed in both neuronal and non-neural tissues, and its expression has been observed in mesenchymal cells of the kidney, tooth enamel and developing sex chord of the testis. Epithelial cells of olfactory, oral, lung and intestine have also shown increased Plexin-B1 expression (Perala et al., 2005). The expression of Plexin-B1, along with VEGFR1, is also found to be lowered in bones of osteoarthritic individuals when compared with normal controls (Hopwood et al., 2005). Expression of Plexin-B1 has also been observed in mouse follicular growth and ovarian maturation (Regev et al., 2005). In another study, a number of different human organs, including kidney, lung and liver showed Plexin-B1 expression in both embryonic as well as adult phases. In contrast to Sema4D expression being present in majority of epithelial cells, the expression of Plexin-B1 is confined mainly to the mesenchyme surrounding epithelial tubules in organs such as the kidney, lung and pancreas. A non redundant role of Plexin-B1 in mouse development has been established (Fazzari et al., 2007). Similarly, branching morphogenesis of ureteric collecting ducts influenced by the Rho-ROCK pathway has also been found to be restricted by Sema4D mediated Plexin-B1 activation (Korostylev et al., 2008). Higher expression of Plexin-B1 and glycodelin were found in human endometrial tissues, with the higher levels being observed during implantation (Amir et al., 2009). Plexin-B1 and Sema4D are involved in a negative feedback loop for maintaining balance among osteoblast and osteoclast cells responsible for bone formation and replenishment (Cao, 2011; Negishi-Koga et al., 2011). Thus, a diversified and promising role for Plexin-B1 in several organs of the body has been reported.

Plexin-B1 involvement in the immune system

In a previous study, expression of Plexin-B1 had been observed in bone marrow stromal cells, dendrocytes and activated T cells of patients with B-cell Chronic Lymphocytic Leukemia (CLL). Under Sema4D stimulation, Plexin-B1 brings about increased proliferation of both CLL and normal CD5+ B cells (Granziero et al., 2003). Interaction of both molecules (Plexin-B1 and CD72) with Sema4D has been reported in the literature (Tamagnone et al., 1999; Kumanogoh and Kikutani, 2003). In contrast to the interaction of Sema4D with Plexin-B1 resulting in enhanced proliferation, interaction of Sema4D with CD72 restricts cell proliferation (Granziero et al., 2003). Reduced expression of Plexin-B1 has also been reported in monocytes, B and T cells during inflammation and these findings were also in line with previously published studies conducted on mouse Sema4D (Kumanogoh and Kikutani, 2001; Ishida et al., 2003). Sema4D mediated stimulation in monocytes can bring about up regulation of pro-inflammatory cytokines. Increased levels of cytokines TNF-alpha, IL-6 and IL8 were observed in endothelial cells when treated with either soluble Sema4D or anti human CD72 agonist affecting cell motility (Chabbert-de Ponnat et al., 2005; Yang et al., 2011). Variation of Plexin-B1expression depends upon numerous factors and types of tissue specificity, nature of transducers and interacting molecules (Bertotti and Comoglio, 2003).

Interaction of Rnd1 (a Rho family member) along with Plexin-B1 is responsible for an increase in RhoA mediated cell motility independent of Sema4D. Similarly, Ras binding with Plexin-B1 is also influenced by Rnd1 interaction with Plexin-B1. Binding of Rnd1 changes the structural topology of Plexin-B1 for mediating, interaction with active rac1, tyrosine

phosphorylation by Her-2 and R-Ras inactivation (Oinuma et al., 2003, 2004; Pasterkamp, 2004). R-Ras GTPase activity is key in inducing growth cone collapse by mediating dephosphorylation of phosphatase and tensin homolog (PTEN). Phosphorylated PTEN is unable to carry out its role in regulating the phosphatidylinositol 3-phosphate (PIP3) level. In a recent report the effect of the interaction of Sema4D and Plexin-B1 on the activation of the PTEN molecule was established and proposed as the main factor responsible for growth cone collapse (Oinuma et al., 2010). Activation of the phosphoinositide 3-kinase (PIP3K) pathway is also among the prerequisites for the initiation of angiogenesis, as it functions downstream of activated RhoA (Binmadi et al., 2011). However, in a study conducted on an ovarian cancer cell line (SKOV3) a reduction of Plexin-B1 expression was found to restrict cell proliferation and migration by inhibiting Akt activity. This finding can be explained by the downstream activation of ErbB-2, to provoke RhoA mediated cell motility, instead of Met, as proposed by Swiercz et al. (2008). Hence, Plexin-B1 based regulation is also influenced by its receptor binding, leading to completely opposite responses.

Role of Plexin-B1 in human uterine cells

Previously, the involvement of Plexin-B1 involvement in cell retraction from the basement membrane and role in cell adhesion has been established. A contrasting role of this molecule in relation to cell adhesiveness has also been observed in other cell lines. The effect of Plexin-B1 on Jar choriocarcinoma cell spheroid adhesion, on both receptive as well as non-receptive endometrial cell lines (RL95-2 and HEC-1A) showed the same findings. A direct correlation of JAR spheroid attachment rate with RL95-2 was observed. However, no direct effect of Plexin-B1 on spheroid growth was observed. Specificity of Plexin-B1 adhesiveness was also confirmed after a forced transfection of Plexin-B1 transgenes in HEC-1A (lacking Plexin-B1 expression). HEC-1A cells showed reduced adhesion when compared with their counterpart transgene cell line retaining Plexin-B1 expressing molecule (Harduf et al., 2007). In a relatively more recent study, implantation of spheroids on the uterine cell lines had also been influenced by both Plexin-B1 and cMet heterodimer cross talk (Harduf et al., 2009). Hence, Plexin-B1 does impart a significant role in endometrial receptivity and in the attachment process of the blastocyts.

Effect of various hormones on Plexin-B1

Based on the hierarchical clustering of gene expression, malignant breast tumours have been classified into four subgroups (luminal A and B, basal-like, normal-like and erbB2-like) (Sorlie et al., 2001). However, in several reports, distinct association of a particular gene expression in relation to a single particular subgroup is lacking and an overlap of genes in expression profiles has been observed (Wang et al., 2005; Ein-Dor et al., 2005; Pau Ni et al., 2012). Only those sets of genes expressing stem cell like (SCL) signatures were identified using microarray analysis. In addition to expressing established stem cell markers (CK5, 6, 14, ITGA6, S100A1, CD24), SCL show both ER positive as well as ER negative states. An inverse correlation between ER state and proliferation was observed in SCL, while a direct correlation of ER positivity with growth was a marked feature of non stem-like cells (Non-SCL). Cluster analysis of 828 ER-regulated genes in both SCL and Non-SCL groups led to the identification of (AHSA2), (RNPC2) and Plexin-B1. Higher expression of Plexin-B1 in ER positive status is correlated with increased disease free and overall survival (van de Vijver et al., 2002). The direct expressional correlation of Plexin-B1 with ER status was observed in only those cancer cells showing stem cell like (SCL) expression status, where proliferative activity was coupled with ER status (Rody et al., 2007). Thus, the Plexin-B1 molecule acts as a mediator responsible for ER mediated restriction of cellular growth. In another study, no association was observed of Plexin-B1 with ER negative samples ($p=0.85$). However, a significant inverse correlation regarding Plexin-B1 with ER status ($p<0.001$) was established in the given dataset ($n=1086$) (Rody et al., 2008). Hence, these studies endorse the earlier findings suggesting Plexin-B1 to have a potential prognostic as well as ER positive correlation.

No significant association between progesterone or testosterone with Sema4D levels has been observed in relation to follicular maturation. The effect of Sema4D and Plexin-B1 in maturation of ovarian follicles significantly correlates with oestradiaol (E2). Though E2 levels were already increasing due to follicle maturation, a peak in the expression can be seen following introduction of Sema4D. Interestingly, introduction of neutralizing antibodies against Plexin-B1 also abolished the E2 surge. This ligand receptor interaction (Sema4D and Plexin-B1) mediates a signalling cascade which influences mitogen activated protein kinases (MAPK) (Regev et al., 2007).

Cross roads among different signalling pathways

Cross talk with c-Met and ErbB-2

ErbB-2 can influence Sema4D Plexin-B1 complex mediated downstream effector response. This was illustrated through the observation that AG1478, an inhibitor of the ErB family, also impaired Plexin-B1 mediated RhoA activity in Cos and human embryonic kidney cells (HEK 293). Interestingly, ErbB2 is involved in inducing phosphorylation of Plexin-B1 and RhoA activation following exposure to Sema4D. However, no regulation of Sema4D via ErbB2 in cells lacking Plexin-B1 expression has been observed. In the same study, it was also observed that PDZ/RhoGEF and ErbB-2

interacting motifs also require Plexin-B1 to mediate cell cytoskeleton rearrangements (Swiercz et al., 2004). In another study, the role of Met as a downstream mediator for Plexin-B1 leading to inactivation of RhoA has also been observed (Giordano et al. 2002). In another study, phosphorylation of Plexin-B1, following Sema4D stimulation, is required for its interaction with Erb-B2. This complex leads to the activation of the Rho molecule. Phosphorylated Plexin-B1 provides a docking site for the phospholipase C gamma (PLCgamma) SH2 domain. This complex is required for activation of the Rho guanine nucleotide exchange factor PDZ-RhoGEF, regulated by the SH3 domain of PLCgamma (Swiercz et al., 2009). In this study, non enzymatic involvement of PLCgamma in the phosphorylation of Plexin-B1 was found to be the factor responsible for RhoGEF mediated cell signalling. Earlier findings regarding c-Met and Her-2 interactions with Plexin-B1 may be attributed to the differences in HEK 293 and MLP29. These contrasting features are also observed in other cell types, when exposed to Sema4D. Sema4D stimulation in neurons results in inhibition of PI3K and Akt signaling (Ito et al., 2006), while in endothelial cells the Akt pathway was activated under Sema4D exposure (Basile et al., 2007a,b). The molecular mechanisms underlying this versatility of Plexin signaling require further full elucidation. It is worthwhile to mention here that the HGF family contains two similar proteins, the Met gene encoding hepatocyte growth factor receptor and the Ron gene encoding macrophage stimulating protein (MSP). The extracellular domain of these factors contains a Sema domain together with a short stretch of 60 amino acids, called the Met related sequence (MRS). The intense binding affinity of these factors mediates tyrosine phosphorylation of all three members of the Plexin-B family leading to invasiveness and tumour cell proliferation (Conrotto et al., 2004; Capparuccia and Tamagnone, 2009). Apart from tissue specific expression of Sema4D and Plexin-B1, autocrine secretions from neighbouring cells in the tumour microenvironment also influence cancer cell adhesion and motility. Similarly, the combined effect of several Semaphorins and other molecules inside the cell result in these contrasting findings.

The role of Plexin-B1 in MAPK and GSK-3 Signalling

Plexin-B1 is also actively involved in the activation of the MAPK signalling pathway. Following Sema4D activation, increased phosphorylation of Plexin-B1 leads to activated ERK. Activation of MAPK signalling requires the presence of the Plexin-B1 C-terminus (Aurandt et al., 2002; Hirotani et al., 2002; Perrot et al., 2002; Swiercz et al., 2002). It has also been shown that the overexpression of LARG can result in RhoA activation. Activated RhoA enhanced ERK activity towards Elk, via the stimulation of a mediator molecule Raf. Hence, the C-terminus of Plexin-B1, Rho-A binding domain, LARG mediated activation and phosphorylation of Raf are all important players of the ERK pathway (Aurandt et al., 2006). Plexin-B1 expression can also induce dephosphorylation of GSK-3, mediating growth cone collapse. Sema4D induced dephosphorylation of Akt or GSK-3beta was not seen in the absence of Rnd1 or Plexin-B1, which similarly highlights its importance in restricting cell proliferation (Ito et al., 2006).

Involvement in cancer and angiogenesis

Plexin-B1 ligand (Sema4D) either as oncogene or tumour suppressor

Immunohistochemical staining of around 700 head and neck squamous cell carcinoma paraffin embedded tissue samples has highlighted Sema4D involvement in disease progression. A significant increase of Sema4D was observed in invading islands of transformed epithelial cells in comparison to normal and non-invasive dysplastic epithelium. This finding was also consistent with previous findings by the same group in oral, prostate, colon, breast and lung cancer tissues. For tumour growth and metastasis progression a persistent vascularisation is required in different types of solid tumours. Recently, down- regulation of Sema4D and Sema4F have also been observed in grade 3 breast tumours when compared with benign tumour tissues (Gabrovska et al., 2011). These contrasting findings are justified on the basis of three main attributes, 1) Involvement of other factors revolving around Semaphorins and their cognate receptors 2) heterogeneity of the cell environment and 3) protective effect of a protoncogene in benign tumours. Increased expression of Plexin-B1 in endothelial cells was found to be responsible for vascularization and stress fibre formation under the influence of stromal Sema4D expression (Basile et al., 2006). Contrary to earlier findings, in melanoma induced by the Raf oncogene, Plexin-B1 expression was found to be significantly down regulated (Argast et al., 2009). Hence, Plexin-B1 is strongly believed to be regulated by mitogen-activated protein kinase signalling in melanoma cells and melanocytes, where it acts as a tumour suppressor in the initial phase of the disease.

Association with matrix metalloproteinases

The pro-angiogenic potential of Sema4D is mediated via its paracrine action on endothelial cells. For paracrine activity, initial cleavage of Sema4D is influenced by metalloproteinases. Matrix metallo-proteinases are zinc dependent endopeptidases responsible for the degradation of the extracellular matrix. Type 1 metalloproteinases specifically target membrane bound proteins and retain peri-cellular proteolytic activity for the processing of membrane bound receptors (Sato et al., 1996). Up-regulated expression of MT1-MMP was significantly related to Sema4D cleavage. In a related set of studies, conducted on head and neck squamous cancer cells, pro-angiogenic potential of Sema4D and Plexin-B1, influenced by MT1-

MMP, has also bee established. As in HN12, cells lacking MT1-MMP expression failed to induce any vascularisation despite the presence of Sema4D (Basile et al., 2006, 2007a,b).

Involvement of Plexin-B1 as an angiogenic marker

Nervous and vascular systems share several anatomical and developmental similarities as both of these systems run together in neurovascular bundles and in peripheral tissues. Interestingly, these shared developmental links also assist scientists to speculate whether there is a similar mechanism of molecular control over the growth and migration of vessels (Carmeliet, 2003; Autiero et al., 2005). Germination of vessels, and their direction guidance in extracellular matrix over great distances to reach their designated targets of innervation, uses a similar analogous process as observed in the nervous system (Dickson and Senti, 2002). Plexins are involved in cardiovascular development enhancing blood vessel formation. In fact, mutations in Plexin-A2, Plexin-D1, Sema3C, Sema3E and NRP1 lead to defective aotic arch and cardiac outflow tract termed DiGeorge and Velocardiofacial syndrome (Gu et al., 2005; Toyofuku et al., 2008). A dual role of Sema4D, either as an angiogenic promoter or suppressor has also been observed. After vascular injury, thrombosis formation, led by platelet occlusion, is mediated by an overall increased expression of Sema4D and its receptors (CD72 and Plexin-B1) on platelets. Furthermore, soluble Sema4D (cleaved from platelets) in addition to interacting with membrane bound Plexin-B1, also communicates with neighbouring endothelial cells to form a plug. This cleavage of membrane associated Sema4D was shown to be induced by metalloprotease ADAM17, also known as tumour necrosis factor (TNF-alpha) (Zhu et al., 2007). These findings were also observed in HNSCC cells where higher levels of soluble Sema4D were seen following the increased expression of MT1-MMP (Basile et al., 2007a,b). Altered actin organization, cell migration and tube formation caused by Sema4D has also been observed in several tumour cell lines (Basile et al., 2004). Sema4D and Plexin-B1 can induce tubule formation and angiogenesis independent of the Met receptor, but do require the PDZ/Rho GEF and the LARG binding motif of Plexin-B1 (Basile et al., 2004). However, no stimulation of any other vascular factors including VEGFA, angiopoietin-2 or HGF was found to be influenced by Sema4D or Plexin-B1 (Conrotto et al., 2005). More interestingly, a recent study revealed a complementary role of Sema4D/Plexin-B1 parallely functioning together with VEGF. It suggests that targeting Sema4D/Plexin-B1 may enhance the anti-angiogenic treatment of targeting VEGF (Zhou et al., 2012a,b). In another study Sema4D stimulated Plexin-B1 was found to elicit an angiogenic phenotype by promoting the formation of focal adhesion complexes, stress fibers and the phosphorylation of myosin light chain through a ROCK mediated pathway (Basile et al., 2007; Sun et al., 2008). In the absence of Sema4D, breast tumour metastasis and angiogenic ability was largely compromised *in vivo*. Interestingly, tumour associated macrophages were identified as a main contributing source of tumour microenvironment proliferation and angiogenesis (Sierra et al., 2008). In addition, the involvement of Sema4A and Plexin-B1 in the progression of atherosclerosis and neovasuclarization in tumour growth has also been observed (Yukawa et al., 2010).

Plexin-B1 involvement in cellular adhesion and motility

Although Plexin-B1 lacks any intrinsic kinase activity, it mediates its effects on cytoskeletal dynamics, integrin mediated cell adhesion and motility using either tyrosine kinase receptors (c-Met, Her-2) or non-tyrosine kinase receptors (pyk2, Src). Semaphorins do regulate integrin expression, although this molecular mechanism requires further research (Serini et al., 2008). Plexin-B1 has been shown to deactivate R-Ras, thus having a direct influence on cell adhesion (Puschel, 2007). Ras activation leads to focal-adhesion formation which influences cell spreading, migration and invasion via PI3K (Negishi et al., 2005).

Plexin-B1, along with Sema4D, exhibits increased cellular motility in endothelial cells through the activation of the tyrosine kinase cascade inside the cells. Phosphorylation of both PYK and Src molecules leads to the activation of the phosphatidylinositol 3-kinase (PI3K)-Akt pathway (Basile et al., 2005). R-Ras, a member of the ras family, involved in cell adhesion, neurite outgrowth and integrin activation is also regulated via binding to Plexin-B1 in the cytoplasm (Negishi et al., 2005). A restricted axon growth pattern of sensory neurons has also been observed in the COS cell line mediated by the influence of Plexin-A1, neuropilin and Plexin-B1 (Turner and Hall, 2006). Sema4D mediated antagonistic effects on integrin (alpha1 and beta1) and R-Ras motility was also influenced by its high affinity receptor Plexin-B1 (Oinuma et al., 2006). Indirect inhibition of PI3-K, a downstream effector of R-Ras, is mediated by this receptor ligand interaction as well as by activating PTEN.

Future prospects

Expression of Plexin-B1 in both cancers of neuronal and non-neuronal origins highlights a multifaceted role of this molecule in cancer progression. The dual nature of Sema4D, acting either as a tumour suppressor or oncogene in different tissues, is largely influenced by Plexin-B1 mediated downstream signalling. Lacking a tyrosine kinase domain, Plexin-B1 acts more like a freelance entity, as studied in relation to different trans-membrane receptors. Depending upon the nature/type of tissue involved, expressional variability has also been observed in relation to both breast and prostate cancer. The effect of oestrogen on the regulation and localization of Plexin-B1 is also one of the limiting

factors yet to be addressed. Due to the heterogeneous nature of cancer, Plexin-B1 along with other members of Semaphorins and Plexins need further studies in relation to cancer. Sema4D and Plexin-B1 are initially characterized as guardians responsible for growth cone collapse, yet their increased expression in relation to different tumours and cancer cell lines is very intriguing. One possible explanation is the heterogeneity and high genetic aberrations of cancer cells. This idea is well supported by the fact that in most of the reports regarding the expression of these molecules the tumours were observed at more advanced stages, particularly with poor differentiation. The impact of Plexin-B1 in tumour angiogenesis provides a novel therapeutic target, as reducing expression of Plexin-B1 consistently shows impaired neovascularization in different cell lines and various *in vivo* models, an area that is still under rigorous investigation.

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References

Amir M., Romano S., Goldman S. and Shalev E. (2009). Plexin-B1, glycodelin and MMP7 expression in the human fallopian tube and in the endometrium. Reprod. Biol. Endocrinol. 7, 152.

Argast G.M., Croy C.H., Couts K.L., Zhang Z., Litman E., Chan D.C. and Ahn N.G. (2009). Plexin B1 is repressed by oncogenic B-Raf signaling and functions as a tumor suppressor inmelanoma cells. Oncogene. 28, 2697-2709.

Artigiani S., Barberis D., Fazzari P., Longati P., Angelini P., van de Loo J.W., Comoglio P.M. and Tamagnone L. (2003). Functional regulation of semaphorin receptors by proprotein convertases. J. Biol. Chem. 278, 10094-10101.

Artigiani S., Conrotto P., Fazzari P., Gilestro G.F., Barberis D., Giordano S., Comoglio P.M. and Tamagnone L. (2004). Plexin-B3 is a functional receptor for Semaphorin 5A. EMBO. Rep. 5, 710-714.

Aurandt J., Vikis H.G., Gutkind J.S., Ahn N. and Guan K.L. (2002). The Semaphorin receptor Plexin-B1 signals through a direct interaction with the Rho-specific nucleotide exchange factor, LARG. Proc. Natl. Acad. Sci. 9, 12085-12090.

Aurandt J., Li W. and Guan K.L. (2006). Semaphorin 4D activates the MAPK pathway downstream of Plexin-B1. Biochem. J. 394, 459-64.

Autiero M., De Smet F. and Claes F.P. (2005). Carmeliet role of neural guidance signals in blood vessel navigation. Cardiovasc. Res. 65, 629-638.

Barberis D., Artigiani S., Casazza A., Corso S., Giordano S., Love C.A., Jones E.Y., Comoglio P.M. and Tamagnone L. (2004). Plexin signaling hampers integrin-based adhesion, leading to Rho-kinase independent cell rounding, and inhibiting lamellipodia extension and cell motility. FASEB. J. 18, 592-594.

Basile J.R., Barac A., Zhu T., Guan K-L. and Gutkind J.S. (2004). Class IV Semaphorins promote angiogenesis by stimulating Rho-initiated pathways through Plexin-B. Cancer Res. 64, 5212-24.

Basile J.R., Afkhami T. and Gutkind J.S. (2005). Semaphorin 4D/plexin-B1 induces endothelial cell migration through the activation of PYK2, Src, and the phosphatidylinositol 3-kinase-Atk pathway. Mol. Cell Biol. 25, 6889-6898.

Basile J.R., Castilho R.M., Williams V.P. and Gutkind J.S. (2006). Semaphorin 4D provides a link between axon guidance processes and tumor induced angiogenesis. Proc. Natl. Acad. Sci. 103, 9017-9022.

Basile J.R., Gavard J. and Gutkind JS. (2007a). Plexin-B1 utilizes RhoA and Rho kinase to promote the integrin dependent activation of Akt and ERK and endothelial cell motility. J. Biol. Chem. 282, 34888-34895.

Basile J.R., Holmbeck K., Bugge T.H. and Gutkind JS (2007b) MT1-MMP controls tumor induced angiogenesis through the release of semaphorin 4D. J. Biol. Chem. 282, 6899-6905.

Bell C.H., Aricescu A.R., Jones E.Y. and Siebold C. (2011). A dual binding mode for RhoGTPases in Plexin signalling. PLoS. Biol. 9, e1001134

Bertotti A. and Comoglio P.M. (2003). Tyrosine kinase signal specificity: Lessons from the HGF receptor. Trends. Biochem. Sci. 28, 527-33.

Billard C., Delaire S., Raffoux E., Bensussan A. and Boumsell L. (2000). Switch in the protein tyrosine phosphatase associated with human CD100 Semaphorin at terminal B-cell differentiation stage. Blood 95, 965-972.

Binmadi N.O., Proia P., Zhou H., Yang Y.H. and Basile J.R. (2011). Rho-mediated activation of PI(4)P5K and lipid second messengers is necessary for promotion ofangiogenesis by Semaphorin 4D. Angiogenesis 14, 309-319.

Bork P., Doerks T., Springer T.A. and Snel B. (1999). Domains in Plexins: links to integrins and transcription factors. Trends Biochem. Sci. 24, 261-263.

Bouguet-Bonnet S. and Buck M. (2008). Compensatory and long-range changes in picosecond-nanosecond main chain dynamics uponcomplex formation: 15N relaxation analysis ofthe free and bound states of the ubiquitin-likedomain of human Plexin-B1 and the small GTPase Rac1. J. Mol. Biol. 377, 1474-1487.

Cao X. (2011). Targeting osteoclast-osteoblast communication. Nat. Med. 17, 1344-1346.

Capparuccia L. and Tamagnone L. (2009). Semaphorin signalling in cancer cells and in cells of the tumor microenvironment--two sides of a coin. J. Cell. Sci. 122, 1723-1736.

Carmeliet P. (2003). Blood vessels and nerves: common signals, pathways and diseases. Nat. Rev. Gen. 4, 710-720.

Chabbert-de Ponnat I., Marie-Cardine A., Pasterkamp R.J., Schiavon V., Tamagnone L., Thomasset N., Bensussan A. and Boumsell L. (2005). Soluble CD100 functions on human monocytes and immature dendritic cells require Plexin C1 and Plexin B1, respectively. Int. Immunol. 17, 439-447.

Chedotal A., Kerjan G. and Moreau-Fauvarque C. (2005). The brain within the tumor: new roles for axon guidance molecules in cancers. Cell. Death. Differ. 8, 1044-1056.

Clarimon J., Scholz S., Fung H.C., Hardy J., Eerola J., Hellstrom O., Chen C.M., Wu Y.R., Tienari P.J. and Singleton A. (2006). Conflicting results regarding the *Semaphorin* gene (SEMA5A) and the risk for Parkinson disease. Am. J. Hum. Genet. 78, 1082-1084.

Comoglio P.M., Tamagnone, L. and Giordano S. (2004). Invasive growth: A two-way street for Semaphorin signalling. Nat. Cell Biol. 6,1155-1157.

Conrotto P., Corso S., Gamberini S., Comoglio P.M. and Giordano S.

(2004). Interplay between scatter factor receptors and B Plexins controls invasive growth. Oncogene 23, 5131-5137.

Conrotto P., Valdembri D., Corso S., Serini G., Tamagnone L., Comoglio P.M., Bussolino F. and Giordano S. (2005). Sema4D induces angiogenesis through Met recruitment by Plexin B1. Blood 105, 4321-4329.

Deaglio S., Vaisitti T., Bergui L., Bonello L., Horenstein A.L., Tamagnone L., Boumsell L. and Malavasi F. (2005). CD38 and CD100 lead a network of surface receptors relaying positive signals for B-CLL growth and survival. Blood 105, 3042-3050.

Deaglio S., Vaisitti T., Aydin S., Ferrero E. and Malavasi F. (2006). In tandem insight from basic science combined with clinical research: CD38 as both marker and key component of the pathogenetic network underlying chronic lymphocytic leukemia. Blood 108, 1135-1144.

Deaglio S., Vaisitti T., Aydin S., Bergui L., D'Arena G., Bonello L., Omedé P., Scatolini M., Jaksic O., Chiorino G., Efremov D. and Malavasi F. (2007) CD38 and ZAP-70 are functionally linked and mark CLL cells with high migratory potential. Blood 110, 4012-4021.

Deng S., Hirschberg A., Worzfeld T., Penachioni J.Y., Korostylev A., Swiercz J.M., Vodrazka P., Mauti O., Stoeckli E.T., Tamagnone L., Offermanns S. and Kuner R. (2007). Plexin-B2, but not Plexin-B1, critically modulates neuronal migration and patterning of the developing nervous system in vivo. J. Neurosci. 27, 6333-6347.

Dickson B.J. and Senti K.A.(2002). Axon guidance: growth cones make an unexpected turn. Curr. Biol. 12, 218-218.

Driessens M.H., Hu H., Nobes C.D., Self A., Jordens I., Goodman C.S. and Hall A. (2001).PlexinB Semaphorin receptors interact directly with active Rac and regulate the actin cytoskeleton by activating Rho. Curr. Biol. 11, 339-344.

Elhabazi. A., Marie-Cardine. A., Chabbert-de Ponnat. I., Bensussan A., Boumsell L. (2003). Structure and function of the immune semaphorin CD100/SEMA4D. Crit. Rev. Immunol. 23, 65-81.

Ein-Dor L., Kela I., Getz G., Givol D. and Domany E. (2005). Outcome signature genes in breast cancer: is there a unique set? Bioinformatics 21, 171-178.

Fazzari P., Penachioni J., Gianola S., Rossi F., Eickholt B.J., Maina F., Alexopoulou L., Sottile A., Comoglio P.M., Flavell R.A. and Tamagnone L. (2007). Plexin B1 plays a redundant role during mouse development and in tumour angiogenesis. BMC Dev. Biol 22, 7-55.

Frost J.A., Khokhlatchev A., Stippec S., White M.A. and Cobb M.H. (1998). Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. J. Biol. Chem. 273, 28191-28198.

Furuyama T., Inagaki S., Kosugi A., Noda S., Saitoh S., Ogata M., Iwahashi Y., Miyazaki N., Hamaoka T. and Tohyama M. (1996). Identification of a novel transmembrane Semaphorin expressed on lymphocytes. J. Biol. Chem. 271, 33376-33381.

Gabrovska P.N., Smith R.A., Tiang T., Weinstein S.R., Haupt L.M. and Griffiths L.R. (2011). Development of an eight gene expression profile implicating human breast tumours of all grade. Mol. Biol. Rep. 39, 3879-3892.

Giacobini P., Messina A., Morello F., Ferraris N., Corso S., Penachioni J., Giordano S., Tamagnone L. and Fasolo A. (2008). Semaphorin 4D regulates gonadotropin hormone-releasing hormone-1 neuronal migration through PlexinB1-Met complex. J. Cell. Biol. 183, 555-566.

Giordano S., Corso S., Conrotto P., Artigiani S., Gilestro G., Barberis D., Tamagnone L. and Comoglio P.M. (2002). The Semaphorin 4D receptor controls invasive growth by coupling with Met. Nat. Cell Biol. 4, 720-724.

Gitler A.D., Lu M.M. and Epstein J.A. (2004). PlexinD1 and semaphorin signaling are required in endothelial cells for cardiovascular development. Dev. Cell 7, 107-116.

Gomez Roman J.J., Garay G.O., Saenz P., Escuredo K., Sanz Ibayondo C., Gutkind S., Junquera C., Simon L., Martinez A., Fernandez Luna J.L., Val-Bernal J.F. (2008). Plexin B1 is downregulated in renal cell carcinomas and modulates cell growth. Transl. Res. 151, 134-140.

Granziero L., Circosta P., Scielzo C., Frisaldi E., Stella S., Geuna M., Giordano S., Ghia P. and Caligaris-Cappio F. (2003). CD100/Plexin-B1 interactions sustain proliferation and survival of normal and leukemic CD5+ B lymphocytes. Blood 101, 1962-1969.

Gu C., Rodriguez E.R., Reimert D.V., Shu T., Fritzsch B., Richards L.J., Kolodkin A.L. and Ginty D.D. (2003). Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev. Cell 5, 45-57.

Gu C., Yoshida Y., Livet J., Reimert D.V., Mann F., Merte J., Henderson C.E., Jessell T.M., Kolodkin A.L. and Ginty D.D. (2005). Semaphorin 3E and Plexin-D1 control vascular pattern independently of neuropilins. Science 307, 265-268.

Hall K.T., Boumsell L., Schultze J.L., Boussiotis V.A., Dorfman D.M., Cardoso A.A., Bensussan A., Nadler L.M. and Freeman G.J. (1996). Human CD100, a novel leukocyte Semaphorin that promotes B-cell aggregation and differentiation Proc. Natl. Acad.Sci. 93, 11780-11785.

Harduf H., Goldman S. and Shalev. (2007). Human uterine epithelial RL95-2 and HEC-1A cell-line adhesiveness: the role of Plexin B1. Fertil. Steril. 87, 1419-27.

Harduf H., Goldman S. and Shalev E. (2009). Progesterone receptor A and c-Met mediates spheroids-endometrium attachment. Reprod. Biol. Endocrinol. 7, 14.

Hartwig C., Veske A., Krejcova S., Rosenberger G. and Finckh U. (2005). Plexin B3 promotes neurite outgrowth, interacts homophilically, and interacts with Rin. BMC Neurosci. 6, 53.

He H., Yang T., Terman J. R. and Zhang X. (2009). Crystal structure of the Plexin A3 intracellular region reveals an autoinhibited conformation through active site sequestration. Proc. Natl. Acad. Sci. USA 106, 15610-15615.

Hirotani M., Ohoka Y., Yamamoto T., Nirasawa H., Furuyama T., Kogo M., Matsuya T. and Inagaki S. (2002). Interaction of Plexin-B1 with PDZ domain-containing Rho guanine nucleotide exchange factors. Biochem. Biophys. Res. Commun. 297, 32-37.

Hirschberg A., Deng S., Korostylev A., Paldy E., Costa M.R., Worzfeld T., Vodrazka P., Wizenmann A., Götz M., Offermanns S. and Kuner R. (2010). Gene deletion mutants reveal a role for semaphorin receptors of the Plexin-B family inmechanisms underlying corticogenesis. Mol. Cell. Biol. 30, 764-80.

Hopwood B., Gronthos S., Kuliwaba J.S., Robey P.G., Findlay D.M. and Fazzalari N.L. (2005). Identification of differentially expressed genes between osteoarthritic and normal trabecular bone from the intertrochanteric region of the proximal femur using cDNA microarray analysis. Bone 6, 635-644.

Hota P.K. and Buck M. (2009). Thermodynamic characterization of two homologous protein complexes: associations of the semaphorin receptor Plexin-B1 RhoGTPase binding domain with Rnd1 and active Rac1. Protein. Sci. 18, 1060-1071.

Hu H., Marton T.F. and Goodman C.S. (2001). Plexin b mediates axon

guidance in Drosophila by simultaneously inhibiting active rac and enhancing RhoA signaling. Neuron 32, 39-51.

Ishida I., Kumanogoh A., Suzuki K., Akahani S., Noda K. and Kikutani H. (2003). Involvement of CD100, a lymphocyte Semaphorin in the activation of the human immune system via CD72: implications for the regulation of immune and inflammatory responses. Int. Immunol. 8, 1027-1034.

Ito Y., Oinuma I., Katoh H., Kaibuchi K. and Negishi M. (2006). Sema4D/Plexin-B1 activates GSK-3beta through R-Ras GAP activity, inducing growth cone collapse. EMBO Rep.7, 704-709.

Janssen B.J., Robinson R.A., Pérez-Brangulí F., Bell C.H., Mitchell K.J., Siebold C. and Jones E.Y. (2010). Structural basis of semaphorin-Plexin signalling. Nature 467, 1118-1122.

Jin Z. and Strittmatter S.M. (1997). Rac1 mediates collapsin-1-induced growth cone collapse. J. Neurosci. 17, 6256-6263.

Kikutani H., Suzuki K. and Kumanogoh A. (2007). Immune semaphorins: increasing members and their diverse roles. Adv. Immunol. 93, 121-143.

Kolodkin A.L., Matthes D.J. and Goodman C.S. (1993). The *Semaphorin* genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell 75, 1389-99.

Korostylev A., Worzfeld T., Deng S., Friedel R.H., Swiercz J.M., Vodrazka P., Maier V., Hirschberg A., Ohoka Y., Inagaki S., Offermanns S. and Kuner R. (2008). A functional role for semaphorin 4D/Plexin B1 interactions in epithelial branching morphogenesis during organogenesis. Development 135, 3333-3343.

Kruger R.P., Aurandt J. and Guan K.L. (2005). Semaphorins command cells to move. Nat. Rev. Mol. Cell. Biol. 6, 789-800.

Kuhn T.B., Brown M.D., Wilcox C.L., Raper J.A. and Bamburg J.R. (1999). Myelin and collapsin-1 induce motor neuron growth cone collapse through different pathways: inhibition of collapse by opposing mutants of Rac1. J. Neurosci. 19, 1965-1975.

Kumanogoh A. and Kikutani H. (2001). The CD100-CD72 interaction: a novel mechanism of immune regulation. Trends Immunol. 22, 670-676.

Kumanogoh A. and Kikutani H. (2003). Roles of the Semaphorin family in immune regulation. Adv. Immunol. 81, 173-198.

Kumanogoh A. and Kikutani H. (2010). Semaphorins and their receptors: Novel features of neural guidance molecules. Proc. Japan. Acad. Series B: Physical and Bio. Sci. 86, 611-620.

Kumanogoh A., Watanabe C., Lee I., Wang X., Shi W., Araki H., Hirata H., Iwahori K., Uchida J., Yasui T., Matsumoto M., Yoshida K., Yakura H., Pan C., Parnes J.R. and Kikutani H. (2000). Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. Immunity 13, 621-631.

Kumanogoh A., Marukawa S., Suzuki K., Takegahara N., Watanabe C., Ch'ng E., Ishida I., Fujimura H., Sakoda S., Yoshida K. and Kikutani H. (2002). Class IV semaphorin Sema4A enhances T-cell activation and interacts with Tim-2. Nature 419, 629-633.

Laht P., Pill K., Haller E., Veske A. (2012). Plexin-B3 interacts with EB-family proteins through a conserved motif. Biochim. Biophys. Acta. 1820, 888-893.

Lalani S.R., Safiullah A.M., Molinari L.M., Fernbach S.D., Martin D.M. and Belmont J.W. (2004). SEMA3E mutation in a patient with CHARGE syndrome. J. Med. Genet. 41, e94.

Lin M.Z. and Greenberg M.E. (2000). Orchestral maneuvers in the axon: Trio and the control of axon guidance. Cell 101, 239-242.

Lin X., Ogiya M., Takahara M., Yamaguchi W., Furuyama T., Tanaka H., Tohyama M. and Inagaki S. (2007). Sema4D-Plexin B1 implicated in regulation of dendritic spine density through RhoA/ROCKpathway. Neurosci. Lett. 428,1-6.

Liu J., Zhang J., Yang Y., Huang H., Shen W., Hu Q., Wang X., Wu J. and Shi Y. (2008). Conformational change upon ligand binding and dynamics of the PDZ domain from leukemia-associated Rho guanine nucleotide exchange factor. Protein. Sci. 17, 1003-1014.

Maestrini E., Tamagnone L., Longati P., Cremona O., Gulisano M., Bione S., Tamanini F., Neel B.G., Toniolo D. and Comoglio P.M. (1996). A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor.Proc. Nat. Acad. Sci. USA 93, 674-678.

Malavasi F., Funaro A., Roggero S., Horenstein A., Calosso L. and Mehta K. (1994). Human CD38: a glycoprotein in search of a function. Immunol. Today 15, 95-97.

Masuda K., Furuyama T., Takahara M., Fujioka S., Kurinami H. and Inagaki S. (2004). Sema4D stimulates axonal outgrowth of embryonic DRG sensory neurones. Genes Cells 9, 821-829.

Moreau-Fauvarque C., Kumanogoh A., Camand E., Jaillard C., Barbin G., Boquet I., Love C., Jones E.Y., Kikutani H., Lubetzki C., Dusart I. and Chédotal A. (2003). The transmembrane Semaphorin Sema4D/CD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J. Neurosci. 23, 9229-9239.

Mou P., Zeng Z., Li Q., Liu X., Xin X., Wannemacher K.M., Ruan C., Li R., Brass L.F. and Zhu L. (2013). Identification of a calmodulin-binding domain in Sema4D that regulates its exodomain shedding in platelets. Blood 121, 4221-4230.

Nagase T., Ishikawa K., Nakajima D., Ohira M., Seki N., Miyajima N., Tanaka A., Kotani H., Nomura N. and Ohara O. (1997). Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. DNA. Res. 4, 141-150.

Negishi M. and Katoh H. (2002). Rho family GTPases as key regulators for neuronal network formation. J. Biochem. 132, 157-166.

Negishi M., Oinuma I. and Katoh H. (2005). Plexins: Axon guidance and signal transduction. Cell. Mol. Life. Sci, 62, 1363-1371.

Negishi M., Oinuma I. and Katoh H. (2005). R-ras as a key player for signaling pathway of Plexins. Mol. Neurobiol. 32, 217-22.

Negishi-Koga T., Shinohara M., Komatsu N., Bito H., Kodama T., Friedel RH. and Takayanagi H. (2011). Suppression of bone formation by osteoclastic expression of semaphorin 4D. Nat. Med. 17, 1473-1480.

Nkyimbeng-Takwi E. and Chapoval S.P. (2011). Biology and function of neuroimmune semaphorins 4A and 4D. Immunol. Res. 50, 10-21.

Ohta K., Mizutani A., Kawakami A., Murakami Y., Kasuya Y., Takagi S., Tanaka H. and Fujisawa H. (1995). Plexin: a novel neuronal cell surface molecule that mediates cell adhesion via a homophilic binding mechanism in the presence of calcium ions. Neuron14, 1189-1199.

Oinuma I., Katoh H., Harada A. and Negishi M. (2003). Direct interaction of Rnd1 with Plexin-B1 regulates PDZ-RhoGEF-mediated Rho activation by PlexinB1 and induces cell contraction in COS7 cells. J. Biol. Chem. 278, 25671-25677.

Oinuma I., Ishikawa Y., Katoh H. and Negishi M. (2004). The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. Science 305, 862-865.

Oinuma I., Katoh H. and Negishi M. (2006). Semaphorin 4D/Plexin-B1-

mediated R-Ras GAP activity inhibits cell migration by regulating beta(1) integrin activity. J. Cell Biol. 173, 601-613.

Oinuma I., Ito Y., Katoh H. and Negishi M. (2010). Semaphorin 4D/Plexin-B1 stimulates PTEN activity through R-Ras GTPase-activating proteinactivity, inducing growth cone collapse in hippocampal neurons. J. Biol. Chem. 285, 28200-28209.

Panizzi J.R., Jessen J.R., Drummond I.A. and Solnica-Krezel L. (2007). New functions for a vertebrate Rho guanine nucleotide exchange factor in ciliated epithelia. Development 134, 921-931.

Pasterkamp R.J. (2004). R-Ras fills another GAP in Semaphorin signalling. Trends Cell. Biol. 2, 61-64.

Pau Ni IB., Zakaria Z., Muhammad R., Abdullah N., Ibrahim N., Aina Emran N., Hisham Abdullah N. and Syed Hussain S.N. (2010). Gene expression patterns distinguish breast carcinomas from normal breast tissues: the Malaysian context. Pathol. Res. Pract. 4, 223-228.

Perala N.M., Immonen T. and Sariola H. (2005). The expression of Plexins during mouse embryogenesis. Gene. Expr. Patterns. 3, 355-62.

Perala N., Sariola H. and Immonen T. (2012). More than nervous: the emerging roles of Plexins. Differentiation 83, 77-91.

Perrot V., Vazquez-Prado J. and Gutkind J.S. (2002). Plexin B regulates Rho through the guanine nucleotide exchange factors leukemia-associated Rho GEF (LARG) and PDZ-RhoGEF. J. Biol. Chem. 277, 43115-43120.

Puschel A.W. (2007). GTPases in semaphorin signaling. Adv. Exp. Med. Biol. 600, 12-23.

Qiang R., Wang F., Shi L.Y., Liu M., Chen S., Wan H.Y., Li Y.X., Li X., Gao S.Y., Sun B.C. and Tang H. (2011). Plexin-B1 is a target of miR 214 in cervical cancer and promotes the growth and invasion of HeLacells. Int. J. Biochem. Cell. Biol. 43, 632-641.

Regev A., Goldman S. and Shalev E. (2005). Expression of Plexin-B1 in the mouse ovary and its possible role in follicular development.. Fertil. Steril. 84, 1210-1219.

Regev A., Goldman S. and Shalev E. (2007). Semaphorin-4D (Sema-4D), the Plexin-B1 ligand, is involved in mouse ovary follicular development. Reprod. Biol. Endocrinol. 21, 5:12.

Rody A., Holtrich U., Gaetje R., Gehrmann M., Engels K., von Minckwitz G., Loibl S., Diallo-Danebrock R., Ruckhäberle E., Metzler D., Ahr A., Solbach C., Karn T. and Kaufmann M. (2007). Poor outcome in estrogen receptor-positive breast cancers predicted by loss of Plexin B-1. Clin. Cancer Res. 13, 1115-1122.

Rody A., Karn T., Holtrich U. and Kaufmann M. (2008). Stem cell like" breast cancers a model for the identification of new prognostic/predictive markers in endocrine responsive breast cancer exemplified by Plexin B1. Eur. J. Obstet. Gynecol. Reprod. Biol. 139, 11-5.

Rohm B., Rahim B., Kleiber B., Hovatta I. and Puschel A.W. (2000) The Semaphorin 3A receptor may directly regulate the activity of small GTPases. FEBS Lett. 486, 68-72.

Rujescu D., Meisenzahl E.M., Krejcova S., Giegling I., Zetzsche T., Reiser M., Born C.M., Möller H.J., Veske A., Gal A. and Finckh U. (2007). Plexin B3 is genetically associated with verbal performance and white matter volume in human brain. Mol. Psychiatr. 12, 190-194.

Saito Y., Oinuma I., Fujimoto S. and Negishi M. (2009). Plexin-B1 is a GTPase activating protein for M-Ras, remodelling dendrite morphology. EMBO Rep. 10, 614-621.

Sato H., Takino T., Kinoshita T., Imai K., Okada Y., Stetler Stevenson W.G. and Seiki M. (1996). Cell surface binding and activation of gelatinase A induced by expression of membrane type 1 matrix metalloproteinase (MT1-MPP). FEBS Lett. 385, 238-240.

Semaphorin Nomenclature Committee. (1999). Unified nomenclature for the semaphorins/collapsins. Cell 97, 551-552.

Serini G., Valdembri D., Zanivan S., Morterra G., Burkhardt C., Caccavari F., Zammataro L., Primo L., Tamagnone L., Logan M., Tessier-Lavigne M., Taniguchi M., Püschel A.W. and Bussolino F. (2003). Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 424, 391-397.

Serini G., Napione L. and Bussolino F. (2008). Integrins team up with tyrosine kinase receptors and Plexins to control angiogenesis. Curr. Opin. Hematol. 15, 235-242.

Shi W., Kumanogoh A., Watanabe C., Lee I., Wang X., Shi W., Araki H., Hirata H., Iwahori K., Uchida J., Yasui T., Matsumoto M., Yoshida K., Yakura H., Pan C., Parnes J.R. and Kikutani H. (2000). Identification of CD72 as a lymphocyte receptor for the class IV Semaphorin CD100: a novel mechanism for regulating B cell signaling. Immunity 5, 621-631.

Shifman M.I. and Selzer M.E. (2007). Differential expression of class 3 and 4 Semaphorins and netrin in the lamprey spinal cord during regeneration. J. Comp. Neurol. 501, 631-646.

Siebold C., Berrow N., Walter T.S., Harlos K., Owens R.J., Stuart D.I., Terman J.R., Kolodkin A.L., Pasterkamp R.J. and Jones E.Y. (2005) Proc. Natl. Acad. Sci. USA 102, 16836-16841.

Sierra J.R., Corso S., Caione L., Cepero V., Conrotto P., Cignetti A., Piacibello W., Kumanogoh A., Kikutani H., Comoglio P.M., Tamagnone L. and Giordano S. (2008). Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associatedmacrophages. J. Exp. Med. 205, 1673-1685.

Sorlie T., Perou C.M., Tibshirani R., Aas T., Geisler S., Johnsen H., Hastie T., Eisen M.B., van de Rijn M., Jeffrey S.S., Thorsen T., Quist H., Matese J.C., Brown P.O., Botstein D., Lønning P.E. and Børresen-Dale A.L. (2001). Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc. Natl. Acad. Sci. USA 98, 10869-10874.

Stevens L., McClelland L., Fricke A., Williamson M., Kuo I. and Scott G. (2010). Plexin B1 suppresses c-Met in melanoma: a role for Plexin B1 as a tumor-suppressor protein through regulation of c-Met. J. Invest. Dermatol. 130, 1636-1645.

Streit W.J., Walter S.A. and Pennel N.A. (1999). Reactive microgliosis. Prog. Neurobiol. 57, 563-581.

Sun Q., Nawabi-Ghasimi F. and Basile J.R. (2008) Semaphorins in vascular development and head and neck squamous cell carcinoma-inducedangiogenesis. Oral. Oncol. 44, 523-531.

Suzuki K., Okuno T., Yamamoto M., Pasterkamp R.J., Takegahara N., Takamatsu H., Kitao T., Takagi J., Rennert P.D., Kolodkin A.L., Kumanogoh A. and Kikutani H. (2007) Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. Nature 446, 680-684.

Swiercz J.M., Kuner R., Behrens J. and Offermanns S. (2002). Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. Neuron 35, 51-63.

Swiercz J.M., Kuner R. and Offermanns S. (2004). Plexin-B1/RhoGEF-mediated RhoA activation involves the receptor tyrosine kinase ErbB-2. J. Cell Biol.165, 869-880.

Swiercz J.M., Worzfeld T. and Offermanns S. (2008). ErbB-2 and met reciprocally regulate cellular signaling via Plexin-B1. J. Biol. Chem. 283, 1893-1901.

Swiercz J.M., Worzfeld T. and Offermanns S. (2009). Semaphorin 4D signaling requires the recruitment of phospholipase C gamma into the Plexin-B1receptor complex. Mol. Cell. Biol. 23, 6321-6334.

Takagi S., Tsuji T., Amagai T., Takamatsu T. and Fujisawa H. (1987). Specific cell surface labels in the visual centers of Xenopus laevis tadpole identified using monoclonal antibodies. Dev. Biol. 122, 90-100.

Tamagnone L., Artigiani S., Chen H., He Z., Ming G.I., Song H., Chedotal A., Winberg M.L., Goodman C.S., Poo M., Tessier-Lavigne M. and Comoglio P.M. (1999). Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored Semaphorins in vertebrates. Cell 99, 71-80.

Toguchi M., Gonzalez D., Furukawa S. and Inagaki S. (2009). Involvement of Sema4D in the control of microglia activation. Neurochem. Int. 55, 573-580.

Toyofuku T., Zhang H., Kumanogoh A., Takegahara N., Yabuki M., Harada K., Hori M. and Kikutani H. (2004). Guidance of myocardial patterning in cardiac development by Sema6D reverse signalling. Nat. Cell Biol. 12, 1204-1211.

Toyofuku T., Yoshida J., Sugimoto T., Yamamoto M., Makino N., Takamatsu H., Takegahara N., Suto F., Hori M., Fujisawa H., Kumanogoh A. and Kikutani H. (2008). Repulsive and attractive Semaphorins cooperate to direct the navigation of cardiac neural crest cells. Dev. Biol. 321, 251-262.

Turner L.J. and Hall A. (2006). Plexin-induced collapse assay in COS cells. Methods Enzymol. 406, 665-676.

Usui H., Taniguchi M., Yokomizo T. and Shimizu T. (2003). Plexin-A1 and Plexin-B1 specifically interact at their cytoplasmic domains. Biochem. Biophys. Res. Commun. 300, 927-31.

van de Vijver M.J., He Y.D., van't Veer L.J., Dai H., Hart A.A., Voskuil D.W., Schreiber G.J., Peterse J.L., Roberts C., Marton M.J., Parrish M., Atsma D., Witteveen A., Glas A., Delahaye L., van der Velde T., Bartelink H., Rodenhuis S., Rutgers E.T., Friend S.H. and Bernards R. (2002). A gene-expression signature as a predictor of survival in breast cancer. N. Engl. J. Med. 347, 1999-2009.

Valente G., Nicotra G., Arrondini M., Castino R., Capparuccia L., Prat M., Kerim S., Tamagnone L. and Isidoro C.. (2009). Co-expression of Plexin-B1 and Met in human breast and ovary tumours enhances the risk of progression. Cell Oncol. 31, 423-436.

Vastrik I., Eichkholt B.J., Walsh F.S., Ridley A. and Doherty P. (1999). Sema3Ainduced growth cone collapse is mediated by Rac1 amino acids 17-32. Curr. Biol. 9, 991-998.

Vikis H.G., Li W., He Z. and Guan K.L. (2000). The Semaphorin receptor Plexin-B1 specifically interacts with active Rac in a ligand-dependent manner. Proc. Natl. Acad. Sci. USA 97, 12457-12462.

Vikis H.G., Li W. and Guan K.L. (2002). The Plexin-B1/Rac interaction inhibits PAK activation and enhances Sema4D ligand binding. Genes. Dev. 16, 836-845.

Vodrazka P., Korostylev A., Hirschberg A., Swiercz J.M., Worzfeld T., Deng S., Fazzari P., Tamagnone L., Offermanns S. and Kuner R. (2009). The Semaphorin 4D-Plexin-B signalling complex regulates dendritic and axonal complexity in developing neurons via diverse pathways. Eur. J. Neurosci. 30, 1193-1208.

Wang Y., Klijn J.G., Zhang Y., Sieuwerts A.M., Look M.P., Yang F., Talantov D., Timmermans M., Meijer-van Gelder M.E., Yu J., Jatkoe T., Berns E.M., Atkins D. and Foekens J.A. (2005). Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 365, 671-679.

Winberg M.L., Noordermeer J.N., Tamagnone L., Comoglio P.M., Spriggs M.K., Tessier-Lavigne M. and Goodman C.S. (1998). Plexin A is a neuronal semaphorin receptor that controls axon guidance. Cell 95, 903-916.

Witherden D.A., Watanabe M., Garijo O., Rieder S.E., Sarkisyan G., Cronin S.J., Verdino P., Wilson I.A., Kumanogoh A., Kikutani H., Teyton L., Fischer W.H.and Havran W.L. (2012). The CD100 receptor interacts with its Plexin B2 ligand to regulate epidermal γδ T cell function. Immunity 24, 314-325.

Wong O.G., Nitkunan T., Oinuma I., Zhou C., Blanc V., Brown R.S., Bott S.R., Nariculam J., Box G., Munson P., Constantinou J., Feneley M.R., Klocker H., Eccles S.A., Negishi M., Freeman A., Masters J.R. and Williamson M. (2007). Plexin-B1 mutations in prostate cancer. Proc. Natl. Acad. Sci. 104, 19040-19045.

Worzfeld T., Rauch P., Karram K., Trotter J., Kuner R. and Offermanns S. (2009). Mice lacking Plexin-B3 display normal CNS morphology and behaviour. Mol. Cell. Neurosci. 42, 372-381.

Worzfeld T., Swiercz J.M., Looso M., Straub B.K., Sivaraj K.K. and Offermanns S. (2012). ErbB-2 signals through Plexin-B1 to promote breast cancer metastasis. J. Clin. Invest. 122, 1296-1305.

Wray N.R., James M.R., Mah S.P., Nelson M., Andrews G., Sullivan P.F., Montgomery G.W., Birley A.J., Braun A. and Martin N.G. (2007). Anxiety and comorbid measures associated with PLXNA2. Arch. Gen. Psychiatry 64, 318-326.

Wu Y., Nadler M.J., Brennan L.A., Gish G.D., Timms J.F., Fusaki N., Jongstra-Bilen J., Tada N., Pawson T., Wither J, Neel B.G. and Hozumi N. (1998). The B-cell transmembrane protein CD72 binds to and is an in vivo substrate of the protein tyrosine phosphatase SHP-1. Curr. Biol. 8, 1009-1017.

Yang Y.H., Zhou H., Binmadi N.O., Proia P. and Basile J.R. (2011). Plexin-B1 activates NF-ÎB and IL-8 to promote a pro-angiogenic response in endothelial cells. PLoS One 6, e25826.

Ye S., Hao X., Zhou T., Wu M., Wei J., Wang Y., Zhou L., Jiang X., Ji L., Chen Y., You L., Zhang Y., Xu G., Zhou J., Ma D. and Wang S. (2010). Plexin-B1 silencing inhibits ovarian cancer cell migration and invasion. BMC Cancer 10, 611.

Yukawa K., Tanaka T., Yoshida K., Takeuchi N., Ito T., Takamatsu H., Kikutani H. and Kumanogoh A. (2010). Sema4A induces cell morphological changes through B-type Plexin-mediated signaling. Int. J. Mol. Med. 25, 225-230.

Zhou C., Wong O.G., Masters J.R. and Williamson M. (2012a). Effect of cancer-associated mutations in the PlexinB1 gene. Mol. Cancer 11, 11.

Zhou H, Binmadi N.O., Yang Y.H., Proia P. and Basile J.R. (2012b) Semaphorin 4D cooperates with VEGF to promote angiogenesis and tumor progression. Angiogenesis 15, 391-407.

Zhu L., Bergmeier W., Wu J., Jiang H., Stalker T.J., Cieslak M., Fan R., Boumsell L., Kumanogoh A., Kikutani H., Tamagnone L., Wagner D.D., Milla M.E. and Brass L.F. (2007). Regulated surface expression and shedding support a dual role for Semaphorin 4D in platelet responses to vascular injury. Proc. Natl. Acad. Sci. USA 104, 1621-1626.

Zielonka M., Xia J., Friedel R.H., Offermanns S. and Worzfeld T. (2010). A systematic expression analysis implicates Plexin-B2 and its ligand Sema4C in the regulation of the vascular and endocrine system. Exp. Cell. Res. 316, 2477-2486.

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