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REVIEW

Class 3 semaphorins in cardiovascular development

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ABSTRACT
Secreted class 3 semaphorins (Sema3), which signal through holoreceptor complexes that are formed by different subunits, such as neuropilins (Nrps), proteoglycans, and plexins, were initially characterized as fundamental regulators of axon guidance during embryogenesis. Subsequently, Sema3A, Sema3C, Sema3D, and Sema3E were discovered to play crucial roles in cardiovascular development, mainly acting through Nrp1 and Plexin D1, which funnels the signal of multiple Sema3 in vascular endothelial cells. Mechanistically, Sema3 proteins control cardiovascular patterning through the enzymatic GTPase-activating-protein activity of the cytodomain of Plexin D1, which negatively regulates the function of Rap1, a small GTPase that is well-known for its ability to drive vascular morphogenesis and to elicit the conformational activation of integrin adhesion receptors.

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The complex morphogenetic events that lead to the development of cardiovascular system, which have been extensively described[1] and/or reviewed[2,3] elsewhere, rely on the property of cells to differentiate, adhere to each other as well as to the surrounding extracellular matrix and migrate in response to guidance cues.[2,3] Among the different molecules capable of regulating the directionality of cell motility, semaphorins (Semas) represent a large family of secreted or membrane-associated glycoproteins, conserved both structurally and functionally from viruses to mammalians and able to provide repulsive or attractive signals to migrating cells.

Sema were originally identified as axon guidance molecules in the developing nervous system.[4,5] Afterward, these molecules have been shown to regulate other physiological and pathological processes outside of the nervous system, such as vascular endothelial cell motility, cardiovascular development, lymphocyte activation, bone and lung morphogenesis, cancer angiogenesis and metastatic dissemination.[3,6,7,8] The Sema family is divided into 8 classes accordingly to structural characteristics and organisms of origin: class 1 and 2 are encoded by invertebrates, classes 3–7 are from vertebrates, and class V Sema are found in viruses. The overall molecular architecture is quite different for the various Sema being characterized by class-specific structural domains. The only exception is the conserved 500 amino acid-long 7-blade β-propeller folded "sema" domain, located close to the N-terminus of the proteins and present in all family members.[9] In vertebrates, class 3 Sema (Sema3) consists of 7 soluble molecules of ~100 kDa (designated by letters from A to G), which are produced as secreted proteins by cells of multiple lineages, including endothelial and epithelial cells, neurons, and specific tumor cells. In Sema3, the N-terminal sema domain is followed by a plexin-semaphorin-integrin (PSI) domain, an immunoglobulin (Ig)-like domain, and a C-terminal basic domain (Fig. 1).

The core components of the Sema3 holoreceptor complexes (Fig. 1) belong to the families of plexins and neuropilins (Nrps) (Table 1). Plexins are a wide family of transmembrane proteins categorized into 4 (A to D) classes on the basis of structural similarities. The extracellular portion of plexins consists of several different moieties, among which a central role is played by a divergent sema domain; their intracellular region contains instead a functionally crucial guanosine triphosphatase (GTPase)–activating protein (GAP) domain[10-13] (Fig. 1). Different Sema crystals have been analyzed so far,[14-18] indicating how all Semas are homodimers, in which, differently from sema domain containing plexins,

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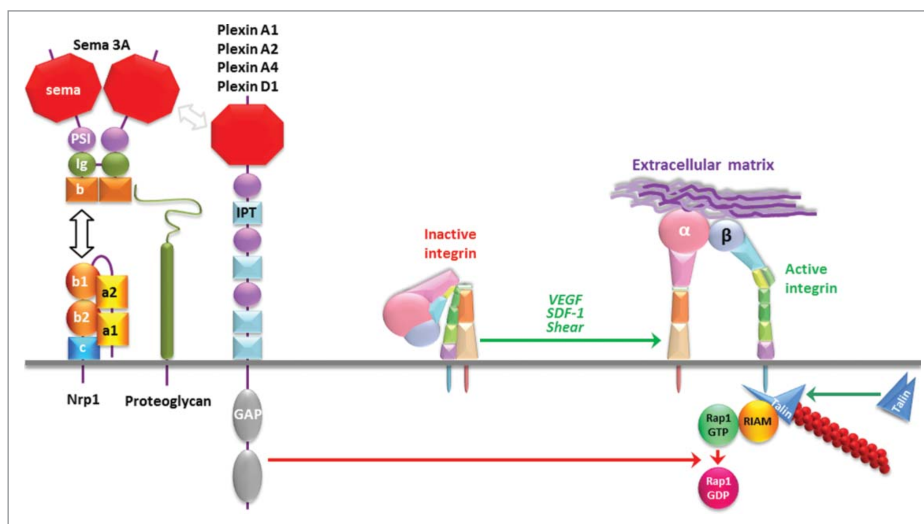

Figure 1. Sema3A signaling *via* the Nrp1-Plexin A/D1 holoreceptor. From the N- to the C-terminus Sema3A displays a sema domain, a PSI domain, an Ig-like domain, and a basic domain. Nrp1 and type A or D plexins constitute the main components of the Sema3A holoreceptor. The extracellular domains of Nrp1 contain 2 complement binding domains (a1/a2), 2 coagulation factor V/VIII homology domains (b1/b2), and a MAM domain (c). The b1 domain of Nrp1 mediates its high affinity (*black double arrow*) binding ~~of Nrps~~ to the basic domain of Sema3A. The extracellular portion of plexins consists of a sema domain and a series of 3 PSI and 4 integrin-transcription factor-plexin (IPT) domains. The intracellular segment of plexins primarily comprises a GAP domain that exerts its enzymatic activity on Rap1, a small GTPase that, *via* effector proteins such as RIAM1, promotes the conformational activation of integrins through talin. The dimeric sema domains of Sema3A would interact at very low affinity (*gray double arrow*) with the sema domains of 2 monomeric type A/D plexins, thus promoting their dimerization (not shown) and the activation of their cytosolic Rap1 GAP enzymatic activity, finally resulting in integrin inactivation.

a 'face-to-face' interaction between the top surfaces of the sema domains occurs.[9] If compared to membrane associated Semas, secreted Sema3 proteins display a less hydrophobic dimer interface that crucially need to be stabilized by disulphide bonds between Ig domains, which are negatively regulated by the proteolytic activity of furins.[19,20] Crystal structures of several membrane associated Semas in complex with their cognate plexin receptors unveiled that electrostatic interactions mediate an head-to-head interaction between each sema domain of a Sema dimer and the sema domain of a monomeric plexin, giving rise to a 2:2 Sema-plexin heterotetramer.[9,15-17] Functional studies provided evidence that the same head-to-head interface is likely employed by

Table 1. Sema3 holoreceptor core components. Nrp co-receptors and plexin receptor that are crucial for transduction of signals elicited by the different Sema3 proteins either in vivo or in vitro are highlighted in bold.

Semaphorin	Neuropilin	References	Plexin	References
Sema3A	Nrp1	90,92,93	Plexin A1, A2, A3, A4, D1	58-63,94,95
Sema3B	Nrp1, Nrp2	79,96	?	
Sema3C	Nrp1, Nrp2	90,96,97	Plexin A2, D1	59,63,81,97-99
Sema3D	Nrp1	100,101	?	
Sema3E	Nrp1	99,102	Plexin D1	64,99,103
Sema3F	Nrp1, Nrp2	26,90	Plexin A1, A2, A3	58-60,95
Sema3G	Nrp1, Nrp2	91,104	?	

Sema3A to bind to and signal through plexin receptors,[17] nevertheless, since so far no physiological high affinity binding has been revealed between the sema domains of Sema3A and plexins,[18] such a canonical binding between Sema3A and plexins must be extremely weak and need the essential involvement of co-receptors such as Nrp1[18] or proteoglycans.[21,22]

In vertebrates 2 Nrps are present (Nrp1 and Nrp2) that act as Sema3 co-receptors.[23] The extracellular domains of both Nrps contain 2 complement binding domains (a1/a2), 2 coagulation factor V/VIII homology domains (b1/b2), and a MAM domain (c), while the short cytoplasmic domain is about 40 amino acids long, and contains a C-terminal 3 amino acid-long (S-E-A) sequence that represents a PDZ-binding motif. In addition to Sema3, Nrp1 and Nrp2 also bind to vascular endothelial growth factor-A (VEGF-A) and -C (VEGF-C) family members respectively and function as their co-receptors.[23-25] The b1 domain mediates the high affinity binding of Nrp1 to the basic domain of Sema3 proteins and VEGF-A.[26-31] While VEGF-A naturally displays a C-terminal arginine, a furin-dependent proteolytic processing of Sema3 must occur to allow the exposure of the Nrp1-binding C-terminal basic sequence.[19,23,30-33] Accordingly, the C-terminal basic stretch peptides of furin-processed Sema3A or Sema3F inhibit effectively and dose-dependently the binding of VEGF-A to the b1

domain of Nrp1.[30,33] Furthermore, 3 independent studies proved that VEGF-A and Sema3A compete for binding Nrp1 on the cell surface and how this competition encompasses a binding site within Nrp1 b1 domain.[34-36] A surface plasmon resonance-based study did not detect any competition between Sema3A and VEGF-A for binding to immobilized Nrp1-Fc [37]; the reason(s) for discrepancies among the work by Appleton et al.[37] and the other 3 studies[34-36] are presently unclear, but they could be due, for example, to differences in furin-cleavage patterning of Sema3A C-terminal basic stretch.[19,33] Indeed, an-N-terminal disulphide-bonded helical region precedes the C-terminal basic stretch of Sema3 proteins[33] and, while the C-terminal basic stretch of Sema3F has only one furin consensus site, Sema3A displays instead 3 furin cleavage sites whose processing is central for Sema3A regulation.[19,33] In particular, shortening the distance between the helical region and the C-terminal motif results in a concomitant reduction of Sema3A affinity for Nrp1 b1 domain[33] and biological activity.[19] The recent finding that proteolytic processing is needed to expose the C-terminal arginine of VEGF-C that directly binds the Nrp2 b1 domain[25] suggests how the binding of Nrp ligands other than Sema3 proteins might also be regulated by the protease-driven strategy. The a1 domain of Nrp1 does not directly bind with high affinity the sema domain of Sema3A,[18] but rather favors the coordination of the latter with the sema domain of type A plexins, such as Plexin A2.[9,18] All together, these data suggest a model in which, while the b1 domain of Nrp1 binds with high affinity to the basic domain of Sema3A, the a1 domain of Nrp1 help the sema domain of Sema3A to coordinate with sema domain of type A plexins and likely activate the signaling of the latter.[9,18,38]

In this review, we summarize the current advances on the involvement of Sema3 in cardiovascular development (Table 2).

Sema3A

In the developing zebrafish embryo, Sema3A is required for the proper patterning of trunk intersegmental blood vessels.[39,40] Gene and/or genome duplication are mechanisms for functional improvement during evolution.[41] Compared to other vertebrate species, the zebrafish teleost ancestor underwent an additional round of whole-genome duplication.[41] As a consequence, the zebrafish displays 2 *Sema3a* ortholog genes, *sema3a1* and *sema3a2* that are expressed in the developing somites.[39] Somite-derived Sema3A1 and Sema3A2 proteins restrain within the intersomitic boundaries the vascular sprouts that bud from trunk large blood vessels. Indeed, *sema3a1*/*sema3a2* and *plxnd1* morphants, as well as the genetic *plxnd1* mutant *out-of-bounds* (*obd*) display inter-segmental blood vessel patterning defects characterized by angiogenic sprouts invading the central region of somites. In addition, Sema3A/PlexinD1 signaling in quiescent aortic ECs adjacent to somites was found to promote the autocrine secretion of a soluble VEGFR1 splice variant capable of sequestering VEGF and restricting blood vessel sprouting to somite boundaries.[40]

Immunohistochemical analysis of the spatial distribution of Sema3A protein in the developing quail embryo was consistent with a negative regulation of vascular patterning.[42] Fittingly, implantation of Sema3A antibody-soaked beads in the developing forelimb of chick embryos caused substantial alterations in the developing vascular pattern; capillaries surrounding the Sema3A antibody-soaked bead were dilated, disorganized, and converged toward the bead.[42] Similarly, retrovirus-mediated delivery of dominant negative constructs of Sema3A holoreceptor components in vascular ECs of the developing chick embryo impaired blood vessel remodeling.[43]

The very few *Sema3a* null mice that survive and go beyond weaning, live longer, and display an altered sympathetic cardiac innervation pattern that results in sinus bradycardia.[44] Cardiac-specific overexpression of *Sema3a* induces a reduction of sympathetic innervation and transgenic animals display susceptibility to ventricular tachycardia.[44] Accordingly, it has been reported that myocardial overexpression of *Sema3a*[45] or intravenous administration of recombinant Sema3A protein[46] after infarction in rats can reduce the probability of ventricular tachycardia that frequently is an associated response to injury, as a result of attenuated sympathetic reinnervation. Moreover, a nonsynonymous polymorphism (I334V, rs138694505A>G) in exon 10 of the human SEMA3A gene was associated with unexplained cardiac arrest and ventricular fibrillation; the axon repelling activity $SEMA3A^{I334V}$ appears significantly weaker of that of its wild type counterpart and in the hearts of patients sympathetic nerves invade the subendocardial layer.[47]

The angiogenic remodeling of both cephalic plexus and dorsal longitudinal anastomotical vessel into mature hierarchically organized vascular trees is severely defective in *Sema3a* knockout embryos.[43] In addition, *Sema3a*$^{-/-}$ pups that survive until the adulthood present an excessive number of glomerular ECs associated with renal vascular defects.[48] The reported lack of vascular abnormalities in one study on *Sema3a* null mice[49] could be due to the use of an age-and-stage matching strategy to compare wild type and *Sema3a* null embryos; indeed, age-and-stage matching inherently overlooks the growth retardation phenotype that, as previously described,[50] usually characterize knockout embryos that display

Table 2. Sema3 and Sema3 receptor mutants with cardiovascular phenotype.

Protein	Animal model	~~Cardiovascular phenotype~~	~~References~~	
Sema3A	Mouse	General ko	Atrial defects, sinus bradycardia, angiogenic remodelling defect of cephalic and dorsal longitudinal vessels, excessive number of glomerular ECs.	43,44,48
			No obvious cardiovascular phenotype	49
		EC specific ko	Increased number and length of filopodia in retinal tip endothelial cells	52
	Zebrafish	Morphants	Inter-segmental blood vessel patterning defects	39,40
	Chicken	Blocking antibodies, dominant-negative receptor constructs	Vascular patterning alterations, vascular remodelling impairment.	42,43
Sema3B	Mouse	General ko	Cardiovascular phenotype not analyzed	79
Sema3C	Mouse	General ko	Improper septation of the cardiac outflow tract, ventricular septal defects, aortic arch defects	82
Sema3D	Mouse	General ko	Anomalous pulmonary venous connection, atrial septal defects, improper patterning of the coronary veins	88,89
Sema3E	Mouse	General ko	Initially severe vascular defects (*e.g.*, in dorsal aortae patterning) that normalize during development	64,65,68
Sema3F	Mouse	General ko	Cardiovascular phenotype not analyzed	105
Sema3G	Mouse	General ko	No obvious cardiovascular phenotype	91
Nrp1	Mouse	General ko	Angiogenic remodelling defects of major head and trunk blood vessels, improper septation of the cardiac outflow tract	56
		Nrp1^{Sema-}	Cardiac defects, lung vascular abnormalities	53-55
		EC specific ko	Brain vasculature abnormalities, reduced branching and vessels interconnections	106
Nrp2	Mouse	General ko	No obvious cardiovascular phenotype	107,108
Nrp1 and Nrp2	Mouse	General ko	Vascular anomalies in embryos and	
~~placenta.~~	~~109~~			
		Nrp1^{Sema-}; *Nrp2*$^{-/-}$	Bilateral atrial enlargement, anomalous origin of the coronary arteries, ventricular septal defect, improper septation of the cardiac outflow tract, no obvious vascular defects	53
Plexin A1	Mouse	General ko	No obvious cardiovascular phenotype	110,111
Plexin A2	Mouse	General ko	Persistent truncus arteriosus and lack of aortic and pulmonary	
~~channel septation with incomplete penetrance.~~	~~112,113~~			
Plexin A2andPlexin A4	Mouse	General ko	Cardiovascular defects with	
~~high penetrance.~~	~~113~~			
Plexin D1	Zebrafish	Morphants and *obd* genetic mutant	Inter-segmental blood vessel patterning defects	39
	Mouse	General ko	Cyanotic after birth, vascular invasion in somite	63
		EC specific ko	Myocardial defects, reduction of bone microvasculature	62

vascular remodeling defects, such as *Sema3a* null mice.[43] Of note, endothelial tip cells of murine retinal vascular spouts were found to express much more *Sema3a* mRNA than stalk ECs,[51] and EC-specific *Sema3a* knockout mice were recently described to exhibit a significantly increased number and length of endothelial tip cell filopodia in retinal vascular sprouts.[52] The latter finding emphasize how paracrine Sema3A secreted by non-vascular cells of adjacent tissues does not rescue the specific function(s) that autocrine EC-derived Sema3A exerts during sprouting angiogenesis.

The role of Nrp1 in Sema3A signaling in ECs appears to be controversial. A *Nrp1*$^{Sema-}$ mouse strain harboring mutations in a1 domain of Nrp1 that finally impair Sema3 protein signaling, at least in neurons, was previously generated.[53] Differently from *Nrp1* null mice, which die by E12.5, 60% of *Nrp1*$^{Sema-}$mouse was originally reported to survive until P7 and to exhibit cardiac, but not vascular abnormalities.[53] However, more recently 2 independent studies[54,55] reported how only 18% of *Nrp1*$^{Sema-}$mouse survive until P4 and present lung vascular abnormalities phenocopying the so-called alveolar capillary dysplasia, *i.e.* severely reduced capillary density, centrally located and dilated alveolar capillaries, hypertensive changes in arteriolar walls, anomalous and misaligned pulmonary veins. However, the lack angiogenic remodeling defects of major head and trunk blood vessels in *Nrp1*$^{Sema-}$ mice[53] and the fact that the vascular phenotype in both Sema3A[43] and Nrp1[56] knockout mice is, on the contrary, highly severe raises the possibility that in mutant *Nrp1*$^{Sema-}$ the responsivity of ECs to Sema3A, albeit reduced, could be, at least in part, maintained due to the existence of additional Sema3A co-receptors other than Nrp1, such as proteoglycans.[21,22] Along this line, it is remarkable that some misprojected axon bundles are present in *Plxna4* null, but neither in *Nrp1*[57] or in ~~*Nrp1*$^{Sema-53}$~~ mutant mice, implying that Plexin A4 may deliver Nrp1-independent Sema3A signals in some neuronal populations.[58] Such a scenario would also be compatible

with the hypothesis that, similarly to membrane associated Semas, Sema3A would directly bind, albeit at very low affinity, and signal *via* plexins.[17] Sema3A has been reported to signal through Plexin A1,[59] Plexin A2,[18,60] Plexin A4,[58,61] and Plexin D1[62] (Table 1). In turn, Plexin D1 was shown to be significantly more efficient that type A plexins in forming high affinity Nrp-dependent holoreceptor complexes for Sema3A and Sema3C.[63] Both Plexin A1 and Plexin A4 were found to be required for Sema3A-elicited collapse of cultured ECs.[61] In addition, aortic ring sprouting assays and Boyden chamber assays revealed how Sema3A inhibits less efficiently the sprouting of aortic blood vessels or the migration of primary ECs isolated from *Plxnd1*$^{-/-}$ than from wild type animals.[62] Therefore, Sema3A may control *in vivo* vascular morphogenesis by binding with high affinity to co-receptors, such as Nrp1 or proteoglycans, and signal through manifold low-affinity receptors, *e.g.* Plexin A1, Plexin A2, Plexin A4 and Plexin D1 (Table 1).

Sema3E

Sema3E binds to high affinity Plexin D1 in a Nrp1-independent manner[64] (Table 1). Both in *Sema3e* and *Plxnd1* knockout embryos blood vessels expand ectopically throughout somites causing the loss of the typical stereotyped intersomitic vascular pattern.[64] However, while *Plxnd1* knockout pups become cyanotic sudden after birth and succumb within 24 hours,[63] *Sema3e*$^{-/-}$ mice are viable, fertile and survive throughout adulthood although displaying initially severe vascular defects,[64,65] thus implying that in the developing embryo Plexin D1 transduces not only the signals of Sema3E, but also those elicited by other Sema3 proteins, such as Sema3A[62] and Sema3C.[63] Interestingly, both Sema3A[66] and Plexin-D1[67] null mice share common axial skeletal defects, such as rib fusion and vertebral split. Moreover, selective endothelial *Tie2-cre*-mediated gene inactivation of *Plxnd1* gene in mice induced myocardial defects and skeletal malformations, associated to a strong reduction of the bone microvasculature.[62] Since Plexin D1 is required for proper blood vessel invasion into the bone, the skeletal defects of *Plxnd1* null mice are most likely secondary to vascular abnormalities.

Sema3E protein produced by the lateral plate mesoderm is required for dorsal aortae patterning and for generating the avascular zones that are located laterally to the dorsal aortae and along the midline.[65,68] During the vasculogenic phase, instead of smooth paired dorsal aortae, *Sema3e*$^{-/-}$ embryos develop highly branched plexiform vessels that, due to unidentified repulsive signal(s) originating from the lateral plate mesoderm convert into single, unbranched dorsal aortae between E8.25 and E8.75.[68] It is anticipated that intersegmental blood vessel patterning defects originally characterized in *Sema3e*$^{-/-}$ embryos[64] are similarly rescued over time by other repulsive guidance cues. Furthermore, differently from zebrafish *sema3a1/sema3a2* and *plexind1* morphants as well as *obd* mutants,[39] the intersomitic blood vessels of *sema3e* zebrafish morphants do not display any angiogenic sprout overshooting phenotype.[69]

Recent studies contributed to shed light on the main pathways that characterize Sema signaling through plexins. The intracellular region of plexins is highly conserved and contains 2 large portions that are highly homologous to Ras GAP domains.[70,71] It has been reported that the Ras GAP-like domain of plexin exert its enzymatic activity on 2 Ras-related small GTPase proteins: R-Ras[72] and M-Ras.[73] However, 2 subsequent studies, albeit reporting a binding between Plexin-D1 or Plexin-B1 and R-Ras, failed to detect any GAP activity toward this small GTPase.[74,75] More recently, Wang and colleagues further confirmed that the purified cytodomains different plexins do not display any GAP activity on R-Ras or M-Ras.[12] Similarly, a recent study on knock-in mice carrying inactivating mutations in the GAP domains of genes encoding for Plexin D1 and Plexin B2 unveiled a crucial R-Ras and M-Ras independent function for the GAP domain of these 2 plexins in the control of the development of nervous, vascular, and skeletal systems.[10] Wang and colleagues provided instead evidence that purified cytoplasmic regions of different plexins exert their GAP activity on the small GTPase Rap1 and that this function was required for plexin-mediated neuronal growth cone collapse[12] (Fig. 1). Subsequently, Wang and colleagues described the crystal structures of zebrafish Plexin C1 cytoplasmic region in complex with Rap1, thus unveiling the conformational changes and molecular details that allow Rap1-binding by plexins.[13] It is well known that Rap1-GTP effectively controls vascular morphogenesis[76] and promotes, *via* talin, the conformational activation of integrins and the ensuing adhesion of different cell types to the extracellular matrix[76,77] (Fig. 1). It is conceivable that both Sema3A and Sema3E inhibit integrin mediated adhesion and promote vascular remodeling[43] by inhibiting Rap1 GTP loading and integrin activation through the GAP activity of plexins.[12,13]

Other Sema3 proteins

Sema3B is as an angiogenesis inhibitor and exerts its effect through the binding to Nrp1[78] (Table 1). Sema3B knockout mice are viable and fertile.[79] An unbiased transcriptomic analysis revealed that in severe forms of human preeclampsia SEMA3B is upregulated in and inhibits the differentiation of placental cytotrophoblasts;

furthermore, cytotrophoblasts-derived SEMA3B may act in a paracrine way to impair uterine microvascular ECs functions.[80]

Sema3C protein binds with high affinity to Nrp1-Plexin D1 and, albeit with lower affinity, to Nrp2-PlexinD1 complexes[63] (Table 1). Accordingly, Sema3C was recently reported to inhibit angiogenesis by signaling *via* Nrp1 and Plexin D1.[81] Deletion of either *Sema3c*[82] or *Nrp1*[56] or *Plxnd1*[63] gene causes postnatal lethality due to cardiovascular defects among which the improper septation of the cardiac outflow tract (OFT), resembling the persistent truncus arteriosus observed in humans.[83] OFT septation depends on the formation, expansion, and fusion of endocardial cushions, finally resulting into a septal bridge; subsequently second heart field-derived smooth muscle cells invade to myocardialize the septum.[84] A recent study proposed that neural crest cell-derived Sema3C elicits the Nrp1-dependent endothelial-to-mesenchymal transition that is needed to give rise to the cell population that form the endocardial cushions; in addition, Sema3C-Nrp1 signaling would also drive septum myocardialization.[85]

Sema3D ~~inhibits~~ EC spreading and migration through a Nrp1 and phosphatidylinositol 3 kinase/Akt dependent pathway[86] (Table 1). Fate mapping studies both in mouse and chick established that Sema3D is expressed in a subpopulation of proepicardial cells that give rise to sinus venosus, a tissue that, at later stages, contributes to the development of the coronary endothelium.[87] Moreover, Sema3D is expressed in the mesocardial reflections that are located between the splanchnic mesoderm and the venous pole of the heart.[88] In the developing embryo, Sema3D would exert a repulsive guidance effect to constrain and to direct pulmonary venous ECs toward the left atrium.[88] Consistently, *Sema3d* null mice exhibit anomalous pulmonary venous connection (APVC) and a c.1806T>A missense mutation that results in the F602L substitution was present in a partial APVC patient.[88] SEMA3D F602L binding to Nrp1 and ability to repel the migration of cultured ECs is significantly reduced.[88] Sema3D was recently reported to be expressed in the left anterior atrioventricular groove to repel venous ECs from aberrantly connecting with the left atrium.[89] It appears that in venous ECs the inhibitory Sema3D signals are conveyed through a Nrp1-ErbB2 holoreceptor complex.[89]

Sema3F binds with high affinity to Nrp2 and, with lower affinity, to Nrp1[90] (Table 1). Although it is well known that Sema3F is an effective inhibitor of cancer angiogenesis (for review see ref. [5]), so far no defects in cardiovascular development were reported in *Sema3f* null mice.

Sema3G binds with high affinity to Nrp2 and, with lower affinity, to Nrp1[91] (Table 1). *Sema3g*$^{-/-}$ mice were reported to be viable and to do not display any obvious vascular phenotype.[91] Sema3G displayed preferential arterial expression in all organs during embryonic development (from E9.5) and postnatally throughout adolescence, while it was downregulated in the adult. Sema3G is produced by ECs and acts as a positive regulator of angiogenic functions both in an autocrine and paracrine way, by promoting smooth muscle cell migration.[91]

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] Walls JR, Coultas L, Rossant J, Henkelman RM. Three-dimensional analysis of vascular development in the mouse embryo. PLoS One 2008; 3:e2853; PMID:18682734; http://dx.doi.org/10.1371/journal.pone.0002853

[2] Herbert SP, Stainier DY. Molecular control of endothelial cell behaviour during blood vessel morphogenesis. Nat Rev Mol Cell Biol 2011; 12:551-64; PMID:21860391; http://dx.doi.org/10.1038/nrm3176

[3] Epstein JA, Aghajanian H, Singh MK. Semaphorin signaling in cardiovascular development. Cell Metab 2015; 21:163-73; PMID:25651171; http://dx.doi.org/10.1016/j.cmet.2014.12.015

[4] Luo Y, Raible D, Raper JA. Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. Cell 1993; 75:217-27; PMID:8402908; http://dx.doi.org/10.1016/0092-8674(93)80064-L

[5] Kolodkin AL, Matthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell 1993; 75:1389-99; PMID:8269517; http://dx.doi.org/10.1016/0092-8674(93)90625-Z

[6] Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. Nat Rev Mol Cell Biol 2005; 6:789-800; PMID:16314868; http://dx.doi.org/10.1038/nrm1740

[7] Neufeld G, Sabag AD, Rabinovicz N, Kessler O. Semaphorins in angiogenesis and tumor progression. Cold Spring Harb Perspect Med 2012; 2:a006718; PMID:22315716; http://dx.doi.org/10.1101/cshperspect.a006718

[8] Serini G, Bussolino F, Maione F, Giraudo E. Class 3 semaphorins: physiological vascular normalizing agents

for anti-cancer therapy. J Intern Med 2013; 273:138-55; PMID:23198760; http://dx.doi.org/10.1111/joim.12017

[9] Siebold C, Jones EY. Structural insights into semaphorins and their receptors. Semin Cell Dev Biol 2013; 24:139-45; PMID:23253452; http://dx.doi.org/10.1016/j.semcdb.2012.11.003

[10] Worzfeld T, Swiercz JM, Sentürk A, Genz B, Korostylev A, Deng S, Xia J, Hoshino M, Epstein JA, Chan AM, et al. Genetic dissection of plexin signaling in vivo. Proc Natl Acad Sci U S A 2014; 111:2194-9; PMID:24469813; http://dx.doi.org/10.1073/pnas.1308418111

[11] Worzfeld T, Offermanns S. Semaphorins and plexins as therapeutic targets. Nat Rev Drug Discov 2014; 13:603-21; PMID:25082288; http://dx.doi.org/10.1038/nrd4337

[12] Wang Y, He H, Srivastava N, Vikarunnessa S, Chen YB, Jiang J, Cowan CW, Zhang X. Plexins are GTPase-activating proteins for Rap and are activated by induced dimerization. Sci Signal 2012; 5:ra6; PMID:22253263

[13] Wang Y, Pascoe HG, Brautigam CA, He H, Zhang X. Structural basis for activation and non-canonical catalysis of the Rap GTPase activating protein domain of plexin. Elife 2013; 2:e01279; PMID:24137545

[14] Antipenko A, Himanen JP, van Leyen K, Nardi-Dei V, Lesniak J, Barton WA, Rajashankar KR, Lu M, Hoemme C, Puschel AW, et al. Structure of the semaphorin-3A receptor binding module. Neuron 2003; 39:589-98; PMID:12925274; http://dx.doi.org/10.1016/S0896-6273(03)00502-6

[15] Liu H, Juo ZS, Shim AH, Focia PJ, Chen X, Garcia KC, He X. Structural basis of semaphorin-plexin recognition and viral mimicry from Sema7A and A39R complexes with PlexinC1. Cell 2010; 142:749-61; PMID:20727575; http://dx.doi.org/10.1016/j.cell.2010.07.040

[16] Janssen BJ, Robinson RA, Pérez-Brangulí F, Bell CH, Mitchell KJ, Siebold C, Jones EY. Structural basis of semaphorin-plexin signalling. Nature 2010; 467:1118-22; PMID:20877282; http://dx.doi.org/10.1038/nature09468

[17] Nogi T, Yasui N, Mihara E, Matsunaga Y, Noda M, Yamashita N, Toyofuku T, Uchiyama S, Goshima Y, Kumanogoh A, et al. Structural basis for semaphorin signalling through the plexin receptor. Nature 2010; 467:1123-7; PMID:20881961; http://dx.doi.org/10.1038/nature09473

[18] Janssen BJ, Malinauskas T, Weir GA, Cader MZ, Siebold C, Jones EY. Neuropilins lock secreted semaphorins onto plexins in a ternary signaling complex. Nat Struct Mol Biol 2012; 19:1293-9; PMID:23104057; http://dx.doi.org/10.1038/nsmb.2416

[19] Adams RH, Lohrum M, Klostermann A, Betz H, Püschel AW. The chemorepulsive activity of secreted semaphorins is regulated by furin-dependent proteolytic processing. EMBO J 1997; 16:6077-86; PMID:9321387; http://dx.doi.org/10.1093/emboj/16.20.6077

[20] Koppel AM, Raper JA. Collapsin-1 covalently dimerizes, and dimerization is necessary for collapsing activity. J Biol Chem 1998; 273:15708-13; PMID:9624167; http://dx.doi.org/10.1074/jbc.273.25.15708

[21] Casazza A, Laoui D, Wenes M, Rizzolio S, Bassani N, Mambretti M, Deschoemaeker S, Van Ginderachter JA, Tamagnone L, Mazzone M. Impeding macrophage entry into hypoxic tumor areas by Sema3A/Nrp1 signaling blockade inhibits angiogenesis and restores antitumor immunity. Cancer Cell 2013; 24:695-709; PMID:24332039; http://dx.doi.org/10.1016/j.ccr.2013.11.007

[22] Pang HB, Braun GB, Ruoslahti E. Neuropilin-1 and heparan sulfate proteoglycans cooperate in cellular uptake of nanoparticles functionalized by cationic cell-penetrating peptides. Sci Adv 2015; 1:e1500821; PMID:26601141; http://dx.doi.org/10.1126/sciadv.1500821

[23] Guo HF, Vander Kooi CW. Neuropilin functions as an essential cell surface receptor. J Biol Chem 2015; 290:29120-6; PMID:26451046; http://dx.doi.org/10.1074/jbc.R115.687327

[24] Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998; 92:735-45; PMID:9529250; http://dx.doi.org/10.1016/S0092-8674(00)81402-6

[25] Parker Matthew W, Linkugel Andrew D, Goel Hira L, Wu T, Mercurio Arthur M, Vander Kooi Craig W. Structural basis for VEGF-C binding to Neuropilin-2 and sequestration by a soluble splice form. Structure 2015; 23:677-87; PMID:25752543; http://dx.doi.org/10.1016/j.str.2015.01.018

[26] Giger RJ, Urquhart ER, Gillespie SK, Levengood DV, Ginty DD, Kolodkin AL. Neuropilin-2 is a receptor for semaphorin IV: insight into the structural basis of receptor function and specificity. Neuron 1998; 21:1079-92; PMID:9856463; http://dx.doi.org/10.1016/S0896-6273(00)80625-X

[27] Nakamura F, Tanaka M, Takahashi T, Kalb RG, Strittmatter SM. Neuropilin-1 extracellular domains mediate semaphorin D/III-induced growth cone collapse. Neuron 1998; 21:1093-100; PMID:9856464; http://dx.doi.org/10.1016/S0896-6273(00)80626-1

[28] Renzi MJ, Feiner L, Koppel AM, Raper JA. A dominant negative receptor for specific secreted semaphorins is generated by deleting an extracellular domain from neuropilin-1. J Neurosci 1999; 19:7870-80; PMID:10479689

[29] Chen H, He Z, Bagri A, Tessier-Lavigne M. Semaphorin-neuropilin interactions underlying sympathetic axon responses to class III semaphorins. Neuron 1998; 21:1283-90; PMID:9883722; http://dx.doi.org/10.1016/S0896-6273(00)80648-0

[30] Parker MW, Xu P, Guo HF, Vander Kooi CW. Mechanism of selective VEGF-A binding by neuropilin-1 reveals a basis for specific ligand inhibition. PLoS One 2012; 7:e49177; PMID:23145112; http://dx.doi.org/10.1371/journal.pone.0049177

[31] Parker MW, Xu P, Li X, Vander Kooi CW. Structural basis for the selective vascular endothelial growth factor-A (VEGF-A) binding to neuropilin-1. J Biol Chem 2012; 287(14):11082-9; PMID:22318724; http://dx.doi.org/10.1074/jbc.M111.331140

[32] Parker MW, Hellman LM, Xu P, Fried MG, Vander Kooi CW. Furin processing of semaphorin 3F determines its anti-angiogenic activity by regulating direct binding and competition for neuropilin. Biochemistry 2010; 49:4068-75; PMID:20387901; http://dx.doi.org/10.1021/bi100327r

[33] Guo HF, Li X, Parker MW, Waltenberger J, Becker PM, Vander Kooi CW. Mechanistic basis for the potent anti-

angiogenic activity of semaphorin 3F. Biochemistry 2013; 52:7551-8; PMID:24079887; http://dx.doi.org/10.1021/bi401034q

[34] Miao HQ, Soker S, Feiner L, Alonso JL, Raper JA, Klagsbrun M. Neuropilin-1 mediates collapsin-1/semaphorin III inhibition of endothelial cell motility: functional competition of collapsin-1 and vascular endothelial growth factor-165. J Cell Biol 1999; 146:233-42; PMID:10402473

[35] Gu C, Limberg BJ, Whitaker GB, Perman B, Leahy DJ, Rosenbaum JS, Ginty DD, Kolodkin AL. Characterization of neuropilin-1 structural features that confer binding to semaphorin 3A and vascular endothelial growth factor 165. J Biol Chem 2002; 277:18069-76; PMID:11886873; http://dx.doi.org/10.1074/jbc.M201681200

[36] Narazaki M, Tosato G. Ligand-induced internalization selects use of common receptor neuropilin-1 by VEGF165 and semaphorin3A. Blood 2006; 107:3892-901; PMID:16424390; http://dx.doi.org/10.1182/blood-2005-10-4113

[37] Appleton BA, Wu P, Maloney J, Yin J, Liang WC, Stawicki S, Mortara K, Bowman KK, Elliott JM, Desmarais W, et al. Structural studies of neuropilin/antibody complexes provide insights into semaphorin and VEGF binding. EMBO J 2007; 26:4902-12; PMID:17989695; http://dx.doi.org/10.1038/sj.emboj.7601906

[38] Parker MW, Guo HF, Li X, Linkugel AD, Vander Kooi CW. Function of members of the neuropilin family as essential pleiotropic cell surface receptors. Biochemistry 2012; 51:9437-46; PMID:23116416; http://dx.doi.org/10.1021/bi3012143

[39] Torres-Vázquez J, Gitler AD, Fraser SD, Berk JD, Van N Pham, Fishman MC, Childs S, Epstein JA, Weinstein BM. Semaphorin-plexin signaling guides patterning of the developing vasculature. Dev Cell 2004; 7:117-23; ; http://dx.doi.org/10.1016/j.devcel.2004.06.008

[40] Zygmunt T, Gay CM, Blondelle J, Singh MK, Flaherty KM, Means PC, Herwig L, Krudewig A, Belting HG, Affolter M, et al. Semaphorin-PlexinD1 signaling limits angiogenic potential via the VEGF decoy receptor sFlt1. Dev Cell 2011; 21:301-14; PMID:21802375; http://dx.doi.org/10.1016/j.devcel.2011.06.033

[41] Meyer A, Schartl M. Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions. Curr Opin Cell Biol 1999; 11:699-704; PMID:10600714; http://dx.doi.org/10.1016/S0955-0674(99)00039-3

[42] Bates D, Taylor GI, Minichiello J, Farlie P, Cichowitz A, Watson N, Klagsbrun M, Mamluk R, Newgreen DF. Neurovascular congruence results from a shared patterning mechanism that utilizes Semaphorin3A and Neuropilin-1. Dev Biol 2003; 255:77-98; PMID:12618135; http://dx.doi.org/10.1016/S0012-1606(02)00045-3

[43] Serini G, Valdembri D, Zanivan S, Morterra G, Burkhardt C, Caccavari F, Zammataro L, Primo L, Tamagnone L, Logan M, et al. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 2003; 424:391-7; PMID:12879061; http://dx.doi.org/10.1038/nature01784

[44] Ieda M, Kanazawa H, Kimura K, Hattori F, Ieda Y, Taniguchi M, Lee JK, Matsumura K, Tomita Y, Miyoshi S, et al. Sema3a maintains normal heart rhythm through sympathetic innervation patterning. Nat Med 2007; 13:604-12; PMID:17417650; http://dx.doi.org/10.1038/nm1570

[45] Chen RH, Li YG, Jiao KL, Zhang PP, Sun Y, Zhang LP, Fong XF, Li W, Yu Y. Overexpression of Sema3a in myocardial infarction border zone decreases vulnerability of ventricular tachycardia post-myocardial infarction in rats. J Cell Mol Med 2013; 17:608-16; PMID:23711091; http://dx.doi.org/10.1111/jcmm.12035

[46] Hu H, Xuan Y, Xue M, Cheng W, Wang Y, Li X, Yin J, Yang N, Shi Y, Yan S. Semaphorin 3A attenuates cardiac autonomic disorders and reduces inducible ventricular arrhythmias in rats with experimental myocardial infarction. BMC Cardiovasc Disord 2016; 16:16; PMID:26787044; http://dx.doi.org/10.1186/s12872-016-0192-8

[47] Nakano Y, Chayama K, Ochi H, Toshishige M, Hayashida Y, Miki D, Hayes CN, Suzuki H, Tokuyama T, Oda N, et al. A nonsynonymous polymorphism in semaphorin 3A as a risk factor for human unexplained cardiac arrest with documented ventricular fibrillation. PLoS Genet 2013; 9:e1003364; PMID:23593010; http://dx.doi.org/10.1371/journal.pgen.1003364

[48] Reidy KJ, Villegas G, Teichman J, Veron D, Shen W, Jimenez J, Thomas D, Tufro A. Semaphorin3a regulates endothelial cell number and podocyte differentiation during glomerular development. Development 2009; 136:3979-89; PMID:19906865; http://dx.doi.org/10.1242/dev.037267

[49] Vieira JM, Schwarz Q, Ruhrberg C. Selective requirements for NRP1 ligands during neurovascular patterning. Development 2007; 134:1833-43; PMID:17428830; http://dx.doi.org/10.1242/dev.002402

[50] Hamada K, Sasaki T, Koni PA, Natsui M, Kishimoto H, Sasaki J, Yajima N, Horie Y, Hasegawa G, Naito M, et al. The PTEN/PI3K pathway governs normal vascular development and tumor angiogenesis. Genes Dev 2005; 19:2054-65; PMID:16107612; http://dx.doi.org/10.1101/gad.1308805

[51] Strasser GA, Kaminker JS, Tessier-Lavigne M. Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. Blood 2010; 115:5102-10; PMID:20154215; http://dx.doi.org/10.1182/blood-2009-07-230284

[52] Ochsenbein AM, Karaman S, Proulx ST, Berchtold M, Jurisic G, Stoeckli ET, Detmar M. Endothelial cell-derived semaphorin 3A inhibits filopodia formation by blood vascular tip cells. Development 2016; 143:589-94; PMID:26884395; http://dx.doi.org/10.1242/dev.127670

[53] Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, Kolodkin AL, Ginty DD. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev Cell 2003; 5:45-57; PMID:12852851; http://dx.doi.org/10.1016/S1534-5807(03)00169-2

[54] Joza S, Wang J, Tseu I, Ackerley C, Post M. Fetal, but not postnatal, deletion of semaphorin-neuropilin-1 signaling affects murine alveolar development. Am J Respir Cell Mol Biol 2013; 49:627-36; PMID:23713442; http://dx.doi.org/10.1165/rcmb.2012-0407OC

[55] Joza S, Wang J, Fox E, Hillman V, Ackerley C, Post M. Loss of semaphorin-neuropilin-1 signaling causes

dysmorphic vascularization reminiscent of alveolar capillary dysplasia. Am J Pathol 2012; 181:2003-17; PMID:23063659; http://dx.doi.org/10.1016/j.ajpath.2012.08.037

[56] Kawasaki T, Kitsukawa T, Bekku Y, Matsuda Y, Sanbo M, Yagi T, Fujisawa H. A requirement for neuropilin-1 in embryonic vessel formation. Development 1999; 126:4895-902; PMID:10518505

[57] Kitsukawa T, Shimizu M, Sanbo M, Hirata T, Taniguchi M, Bekku Y, Yagi T, Fujisawa H. Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice. Neuron 1997; 19:995-1005; PMID:9390514; http://dx.doi.org/10.1016/S0896-6273(00)80392-X

[58] Yaron A, Huang PH, Cheng HJ, Tessier-Lavigne M. Differential requirement for Plexin-A3 and -A4 in mediating responses of sensory and sympathetic neurons to distinct class 3 Semaphorins. Neuron 2005; 45:513-23; PMID:15721238; http://dx.doi.org/10.1016/j.neuron.2005.01.013

[59] Takahashi T, Fournier A, Nakamura F, Wang LH, Murakami Y, Kalb RG, Fujisawa H, Strittmatter SM. Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. Cell 1999; 99:59-69; PMID:10520994; http://dx.doi.org/10.1016/S0092-8674(00)80062-8

[60] Takahashi T, Strittmatter SM. Plexina1 autoinhibition by the plexin sema domain. Neuron 2001; 29:429-39; PMID:11239433; http://dx.doi.org/10.1016/S0896-6273(01)00216-1

[61] Kigel B, Rabinowicz N, Varshavsky A, Kessler O, Neufeld G. Plexin-A4 promotes tumor progression and tumor angiogenesis by enhancement of VEGF and bFGF signaling. Blood 2011; 118:4285-96; PMID:21832283; http://dx.doi.org/10.1182/blood-2011-03-341388

[62] Zhang Y, Singh MK, Degenhardt KR, Lu MM, Bennett J, Yoshida Y, Epstein JA. Tie2Cre-mediated inactivation of plexinD1 results in congenital heart, vascular and skeletal defects. Dev Biol 2009; 325:82-93; PMID:18992737; http://dx.doi.org/10.1016/j.ydbio.2008.09.031

[63] Gitler AD, Lu MM, Epstein JA. PlexinD1 and semaphorin signaling are required in endothelial cells for cardiovascular development. Dev Cell 2004; 7:107-16; PMID:15239958; http://dx.doi.org/10.1016/j.devcel.2004.06.002

[64] Gu C, Yoshida Y, Livet J, Reimert DV, Mann F, Merte J, Henderson CE, Jessell TM, Kolodkin AL, Ginty DD. Semaphorin 3E and plexin-D1 control vascular pattern independently of neuropilins. Science 2005; 307:265-8; PMID:15550623; http://dx.doi.org/10.1126/science.1105416

[65] Meadows SM, Fletcher PJ, Moran C, Xu K, Neufeld G, Chauvet S, Mann F, Krieg PA, Cleaver O. Integration of repulsive guidance cues generates avascular zones that shape mammalian blood vessels. Circ Res 2012; 110:34-46; PMID:22076636; http://dx.doi.org/10.1161/CIRCRESAHA.111.249847

[66] Behar O, Golden JA, Mashimo H, Schoen FJ, Fishman MC. Semaphorin III is needed for normal patterning and growth of nerves, bones and heart. Nature 1996; 383:525-8; PMID:8849723; http://dx.doi.org/10.1038/383525a0

[67] Kanda T, Yoshida Y, Izu Y, Nifuji A, Ezura Y, Nakashima K, Noda M. PlexinD1 deficiency induces defects in axial skeletal morphogenesis. J Cell Biochem 2007; 101:1329-37; PMID:17477353; http://dx.doi.org/10.1002/jcb.21306

[68] Meadows SM, Ratliff LA, Singh MK, Epstein JA, Cleaver O. Resolution of defective dorsal aortae patterning in Sema3E-deficient mice occurs via angiogenic remodeling. Dev Dyn 2013; 242:580-90; PMID:23444297; http://dx.doi.org/10.1002/dvdy.23949

[69] Lamont RE, Lamont EJ, Childs SJ. Antagonistic interactions among Plexins regulate the timing of intersegmental vessel formation. Dev Biol 2009; 331:199-209; PMID:19422817; http://dx.doi.org/10.1016/j.ydbio.2009.04.037

[70] Rohm B, Rahim B, Kleiber B, Hovatta I, Püschel AW. The semaphorin 3A receptor may directly regulate the activity of small GTPases. FEBS Lett 2000; 486:68-72; PMID:11108845; http://dx.doi.org/10.1016/S0014-5793(00)02240-7

[71] Hu H, Marton TF, Goodman CS. Plexin B mediates axon guidance in Drosophila by simultaneously inhibiting active Rac and enhancing RhoA signaling. Neuron 2001; 32:39-51; PMID:11604137; http://dx.doi.org/10.1016/S0896-6273(01)00453-6

[72] Oinuma I, Ishikawa Y, Katoh H, Negishi M. The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. Science 2004; 305:862-5; PMID:15297673; http://dx.doi.org/10.1126/science.1097545

[73] Saito Y, Oinuma I, Fujimoto S, Negishi M. Plexin-B1 is a GTPase activating protein for M-Ras, remodelling dendrite morphology. EMBO Rep 2009; 10:614-21; PMID:19444311; http://dx.doi.org/10.1038/embor.2009.63

[74] Sakurai A, Gavard J, Annas-Linhares Y, Basile JR, Amornphimoltham P, Palmby TR, Yagi H, Zhang F, Randazzo PA, Li X, et al. Semaphorin 3E initiates antiangiogenic signaling through plexin D1 by regulating Arf6 and R-Ras. Mol Cell Biol 2010; 30:3086-98; PMID:20385769; http://dx.doi.org/10.1128/MCB.01652-09

[75] Tong Y, Hota PK, Penachioni JY, Hamaneh MB, Kim S, Alviani RS, Shen L, He H, Tempel W, Tamagnone L, et al. Structure and function of the intracellular region of the plexin-b1 transmembrane receptor. J Biol Chem 2009; 284:35962-72; PMID:19843518; http://dx.doi.org/10.1074/jbc.M109.056275

[76] Chrzanowska-Wodnicka M. Distinct functions for Rap1 signaling in vascular morphogenesis and dysfunction. Exp Cell Res 2013; 319:2350-9; PMID:23911990; http://dx.doi.org/10.1016/j.yexcr.2013.07.022

[77] Calderwood DA, Campbell ID, Critchley DR. Talins and kindlins: partners in integrin-mediated adhesion. Nat Rev Mol Cell Biol 2013; 14:503-17; PMID:23860236; http://dx.doi.org/10.1038/nrm3624

[78] Varshavsky A, Kessler O, Abramovitch S, Kigel B, Zaffryar S, Akiri G, Neufeld G. Semaphorin-3B is an angiogenesis inhibitor that is inactivated by furin-like pro-protein convertases. Cancer Res 2008; 68:6922-31; PMID:18757406; http://dx.doi.org/10.1158/0008-5472.CAN-07-5408

[79] Falk J, Julien F, Bechara A, Fiore R, Nawabi H, Zhou H, Hoyo-Becerra C, Bozon M, Rougon G, Grumet M, et al. Dual functional activity of semaphorin 3B is required for positioning the anterior commissure. Neuron 2005; 48:63-75; PMID:16202709; http://dx.doi.org/10.1016/j.neuron.2005.10.024

825 [80] Zhou Y, Gormley MJ, Hunkapiller NM, Kapidzic M, Stolyarov Y, Feng V, Nishida M, Drake PM, Bianco K, Wang F, et al. Reversal of gene dysregulation in cultured cytotrophoblasts reveals possible causes of preeclampsia. J Clin Invest 2013; 123:2862-72; PMID:23934129;
830 http://dx.doi.org/10.1172/JCI66966
[81] Yang WJ, Hu J, Uemura A, Tetzlaff F, Augustin HG, Fischer A. Semaphorin-3C signals through Neuropilin-1 and PlexinD1 receptors to inhibit pathological angiogenesis. EMBO Mol Med 2015; 7:1267-84; PMID:26194913;
835 http://dx.doi.org/10.15252/emmm.201404922
[82] Feiner L, Webber AL, Brown CB, Lu MM, Jia L, Feinstein P, Mombaerts P, Epstein JA, Raper JA. Targeted disruption of semaphorin 3C leads to persistent truncus arteriosus and aortic arch interruption. Devel-
840 opment 2001; 128:3061-70; PMID:11688556
[83] Crupi G, Macartney FJ, Anderson RH. Persistent truncus arteriosus. A study of 66 autopsy cases with special reference to definition and morphogenesis. Am J Cardiol 1977; 40:569-78; PMID:910720; http://dx.doi.org/
845 10.1016/0002-9149(77)90073-X
[84] Sugishita Y, Watanabe M, Fisher SA. The development of the embryonic outflow tract provides novel insights into cardiac differentiation and remodeling. Trends Cardiovasc Med 2004; 14:235-41; PMID:15451515;
850 http://dx.doi.org/10.1016/j.tcm.2004.06.004
[85] Plein A, Calmont A, Fantin A, Denti L, Anderson NA, Scambler PJ, Ruhrberg C. Neural crest-derived SEMA3C activates endothelial NRP1 for cardiac outflow tract septation. J Clin Invest 2015; 125:2661-76;
855 PMID:26053665; http://dx.doi.org/10.1172/JCI79668
[86] Aghajanian H, Choi C, Ho VC, Gupta M, Singh MK, Epstein JA. Semaphorin 3d and semaphorin 3e direct endothelial motility through distinct molecular signaling pathways. J Biol Chem 2014; 289:17971-9; PMID:24825896;
860 http://dx.doi.org/10.1074/jbc.M113.544833
[87] Katz TC, Singh MK, Degenhardt K, Rivera-Feliciano J, Johnson RL, Epstein JA, Tabin CJ. Distinct compartments of the proepicardial organ give rise to coronary vascular endothelial cells. Dev Cell 2012;
865 22:639-50; PMID:22421048; http://dx.doi.org/10.1016/j.devcel.2012.01.012
[88] Degenhardt K, Singh MK, Aghajanian H, Massera D, Wang Q, Li J, Li L, Choi C, Yzaguirre AD, Francey LJ, et al. Semaphorin 3d signaling defects are associated
870 with anomalous pulmonary venous connections. Nat Med 2013; 19:760-5; PMID:23685842; http://dx.doi.org/10.1038/nm.3185
[89] Aghajanian H, Cho YK, Manderfield LJ, Herling MR, Gupta M, Ho VC, Li L, Degenhardt K, Aharonov A,
875 Tzahor E, et al. Coronary vasculature patterning requires a novel endothelial ErbB2 holoreceptor. Nat Commun 2016; 7:12038; PMID:27356767; http://dx.doi.org/10.1038/ncomms12038
[90] Chen H, Chedotal A, He Z, Goodman CS, Tessier-
880 Lavigne M. Neuropilin-2, a novel member of the neuropilin family, is a high affinity receptor for the semaphorins Sema E and Sema IV but not Sema III. Neuron 1997; 19:547-59; PMID:9331348; http://dx.doi.org/10.1016/S0896-6273(00)80371-2
885 [91] Kutschera S, Weber H, Weick A, De Smet F, Genove G, Takemoto M, Prahst C, Riedel M, Mikelis C, Baulande S, et al. Differential endothelial transcriptomics identifies semaphorin 3G as a vascular class 3 semaphorin. Arterioscler Thromb Vasc Biol 2011; 31:151-9; PMID:20947821; http://dx.doi.org/10.1161/ATVBAHA.110.215871 890
[92] He Z, Tessier-Lavigne M. Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. Cell 1997; 90:739-51; PMID:9288753; http://dx.doi.org/10.1016/S0092-8674(00)80534-6
[93] Kolodkin AL, Levengood DV, Rowe EG, Tai YT, Giger 895 RJ, Ginty DD. Neuropilin is a semaphorin III receptor. Cell 1997; 90:753-62; PMID:9288754; http://dx.doi.org/10.1016/S0092-8674(00)80535-8
[94] Tamagnone L, Artigiani S, Chen H, He Z, Ming GI, Song H, Chedotal A, Winberg ML, Goodman CS, Poo 900 M, et al. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell 1999; 99:71-80; PMID:10520995; http://dx.doi.org/10.1016/S0092-8674(00)80063-X
[95] Cheng HJ, Bagri A, Yaron A, Stein E, Pleasure SJ, Tessier- 905 Lavigne M. Plexin-A3 mediates semaphorin signaling and regulates the development of hippocampal axonal projections. Neuron 2001; 32:249-63; PMID:11683995; http://dx.doi.org/10.1016/S0896-6273(01)00478-0
[96] Takahashi T, Nakamura F, Jin Z, Kalb RG, Strittmatter SM. 910 Semaphorins A and E act as antagonists of neuropilin-1 and agonists of neuropilin-2 receptors. Nat Neurosci 1998; 1:487-93; PMID:10196546; http://dx.doi.org/10.1038/2203
[97] Rohm B, Ottemeyer A, Lohrum M, Puschel AW. Plexin/neuropilin complexes mediate repulsion by the axonal 915 guidance signal semaphorin 3A. Mech Dev 2000; 93:95-104; PMID:10781943; http://dx.doi.org/10.1016/S0925-4773(00)00269-0
[98] Brown CB, Feiner L, Lu MM, Li J, Ma X, Webber AL, Jia L, Raper JA, Epstein JA. PlexinA2 and semaphorin sig- 920 naling during cardiac neural crest development. Development 2001; 128:3071-80; PMID:11688557
[99] Chauvet S, Cohen S, Yoshida Y, Fekrane L, Livet J, Gayet O, Segu L, Buhot MC, Jessell TM, Henderson CE, et al. Gating of Sema3E/PlexinD1 signaling by neuropilin-1 925 switches axonal repulsion to attraction during brain development. Neuron 2007; 56:807-22; PMID:18054858; http://dx.doi.org/10.1016/j.neuron.2007.10.019
[100] Feiner L, Koppel AM, Kobayashi H, Raper JA. Secreted chick semaphorins bind recombinant neuropilin with 930 similar affinities but bind different subsets of neurons in situ. Neuron 1997; 19:539-45; PMID:9331347; http://dx.doi.org/10.1016/S0896-6273(00)80370-0
[101] Wolman MA, Liu Y, Tawarayama H, Shoji W, Halloran MC. Repulsion and attraction of axons by Semaphor- 935 in3D are mediated by different neuropilins in vivo. J Neurosci 2004; 24:8428-35; PMID:15456815; http://dx.doi.org/10.1523/JNEUROSCI.2349-04.2004
[102] Miyazaki N, Furuyama T, Sakai T, Fujioka S, Mori T, Ohoka Y, Takeda N, Kubo T, Inagaki S. Developmental 940 localization of semaphorin H messenger RNA acting as a collapsing factor on sensory axons in the mouse brain. Neuroscience 1999; 93:401-8; PMID:10430503; http://dx.doi.org/10.1016/S0306-4522(99)00134-7
[103] Casazza A, Finisguerra V, Capparuccia L, Camperi A, 945 Swiercz JM, Rizzolio S, Rolny C, Christensen C, Bertotti A, Sarotto I, et al. Sema3E-Plexin D1 signaling drives human cancer cell invasiveness and metastatic

spreading in mice. J Clin Invest 2010; 120:2684-98; PMID:20664171; http://dx.doi.org/10.1172/JCI42118

[104] Taniguchi M, Masuda T, Fukaya M, Kataoka H, Mishina M, Yaginuma H, Watanabe M, Shimizu T. Identification and characterization of a novel member of murine semaphorin family. Genes Cells 2005; 10:785-92; PMID:16098142; http://dx.doi.org/10.1111/j.1365-2443.2005.00877.x

[105] Sahay A, Molliver ME, Ginty DD, Kolodkin AL. Semaphorin 3F is critical for development of limbic system circuitry and is required in neurons for selective CNS axon guidance events. J Neurosci 2003; 23:6671-80; PMID:12890759

[106] Fantin A, Vieira JM, Plein A, Denti L, Fruttiger M, Pollard JW, Ruhrberg C. NRP1 acts cell autonomously in endothelium to promote tip cell function during sprouting angiogenesis. Blood 2013; 121:2352-62; PMID:23315162; http://dx.doi.org/10.1182/blood-2012-05-424713

[107] Giger RJ, Cloutier JF, Sahay A, Prinjha RK, Levengood DV, Moore SE, Pickering S, Simmons D, Rastan S, Walsh FS, et al. Neuropilin-2 is required in vivo for selective axon guidance responses to secreted semaphorins. Neuron 2000; 25:29-41; PMID:10707970; http://dx.doi.org/10.1016/S0896-6273(00)80869-7

[108] Yuan L, Moyon D, Pardanaud L, Breant C, Karkkainen MJ, Alitalo K, Eichmann A. Abnormal lymphatic vessel development in neuropilin 2 mutant mice. Development 2002; 129:4797-806; PMID:12361971

[109] Takashima S, Kitakaze M, Asakura M, Asanuma H, Sanada S, Tashiro F, Niwa H, Miyazaki Ji J, Hirota S, Kitamura Y, et al. Targeting of both mouse neuropilin-1 and neuropilin-2 genes severely impairs developmental yolk sac and embryonic angiogenesis. Proc Natl Acad Sci U S A 2002; 99:3657-62; PMID:11891274; http://dx.doi.org/10.1073/pnas.022017899

[110] Bouvrée K, Brunet I, Del Toro R, Gordon E, Prahst C, Cristofaro B, Mathivet T, Xu Y, Soueid J, Fortuna V, et al. Semaphorin3A, Neuropilin-1, and PlexinA1 are required for lymphatic valve formation. Circ Res 2012; 111:437-45; http://dx.doi.org/10.1161/CIRCRESAHA.112.269?316

[111] Takegahara N, Takamatsu H, Toyofuku T, Tsujimura T, Okuno T, Yukawa K, Mizui M, Yamamoto M, Prasad DV, Suzuki K, et al. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. Nat Cell Biol 2006; 8:615-22; PMID:16715077; http://dx.doi.org/10.1038/ncb1416

[112] Suto F, Tsuboi M, Kamiya H, Mizuno H, Kiyama Y, Komai S, Shimizu M, Sanbo M, Yagi T, Hiromi Y, et al. Interactions between plexin-A2, plexin-A4, and semaphorin 6A control lamina-restricted projection of hippocampal mossy fibers. Neuron 2007; 53:535-47; PMID:17296555; http://dx.doi.org/10.1016/j.neuron.2007.01.028

[113] Toyofuku T, Yoshida J, Sugimoto T, Yamamoto M, Makino N, Takamatsu H, Takegahara N, Suto F, Hori M, Fujisawa H, et al. Repulsive and attractive semaphorins cooperate to direct the navigation of cardiac neural crest cells. Dev Biol 2008; 321:251-62; PMID:18625214; http://dx.doi.org/10.1016/j.ydbio.2008.06.028