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REVIEW

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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.

Semas 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 Semas, 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]

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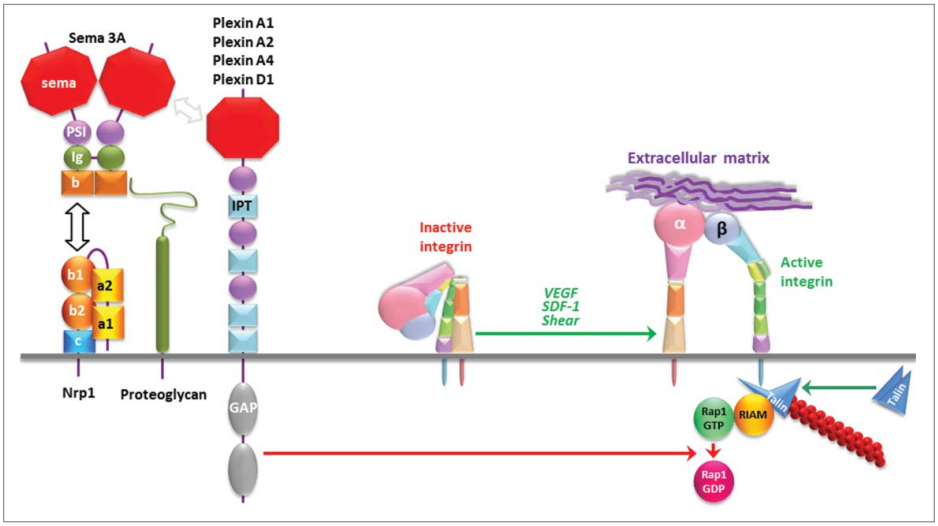

Figure 1. Sema3A signaling *via* the Nrp1-Plexin A/D1 holoreceptor. From the N- to the C-terminus Sema3A displays a sema domain, a PSI domain, an Ig-like domain, and a basic domain. Nrp1 and type A or D plexins constitute the main components of the Sema3A holoreceptor. The extracellular domains of Nrp1 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 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.

(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, 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 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]

Table 1. Sema3 holoreceptor core components.

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	?	

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.

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,

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

Protein	Animal model	Experimental strategy	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 A2 and Plexin 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

age-and-stage matching inherently overlooks the growth retardation phenotype that, as previously described,[50] usually characterize knockout embryos that display 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 than 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 with high affinity to 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 of 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 EC 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

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