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Davide Corà[1, 2], Elena Astanina[1, 2], Enrico Giraudo[3, 4], Federico Bussolino[1, 2,]

[1] Department of Oncology, University of Torino, Torino, Italy

[2] Candiolo Cancer Institute, Torino, Candiolo, Italy

[3] Candiolo Cancer Institute-FPO, IRCCS, Torino, Candiolo, Italy

[4] Department of Science and Drug Technology, University of Torino, Torino, Italy

During organogenesis, patterning is primarily achieved by the combined actions of morphogens. Among these, semaphorins represent a general system for establishing the appropriate wiring architecture of biological nets. Originally discovered as evolutionarily conserved steering molecules for developing axons, subsequent studies on semaphorins expanded their functions to the cardiovascular and immune systems. Semaphorins participate in cardiac organogenesis and control physiological vasculogenesis and angiogenesis, which result from a balance between pro- and anti-angiogenic signals. These signals are altered in several diseases. In this review, we discuss the role of semaphorins in vascular biology, emphasizing the mechanisms by which these molecules control vascular patterning and lymphangiogenesis, as well as in genetically inherited and degenerative vascular diseases.

Keywords
angiogenesis;
plexins;
endothelial cells;
cardiac diseases;
vascular diseases

The semaphorin protein family

Semaphorins (SEMAs) are a family of secreted and membrane-associated molecules that were originally identified as regulators of growth cone guidance in the grasshopper embryo [1]. Subsequently, SEMAs emerged as regulators of a wide range of biological functions. The SEMA family consists of seven classes: classes 1 and 2 are present in invertebrates, whereas classes 3–7 are unique to vertebrates. SEMAs can be secreted (classes 2 and 3), cross the plasma membrane (classes 1, 4, 5, and 6), or be linked to a glycosylphosphatidylinositol-bound membrane protein (class 7).

SEMAs are characterized by an ∼500-amino acid-conserved SEMA domain common to all family members and, with the exception of certain viral semaphorins, by a short plexin–semaphorin–integrin (PSI) domain. Semaphorins are further distinguished by other specific protein domains, including immunoglobulin-like (classes 3, 4, and 7), thrombospondin (class 5), immunoglobulin domains shared by plexins and transcription factors (class 6), and basic C-terminal domains (class 3) 2 and 3. SEMA activities are mediated by plexin receptors, which consist of four subfamilies: PLEXIN-A (1–4), -B (1–3), -C1, and -D. Neuropilins (NRPs), tyrosine kinase receptors, heparan sulfates, and several types of adhesive molecules, scaffolds, and adaptors can cooperate with plexins as co-receptors. In some cases, they act as ligand-binding units of SEMA receptor complexes. Alternatively, they also serve as modulators of plexins in a cell-specific manner, and can mediate distinctive signaling cascades, leading to divergent biological outcomes2 and 3. With the exception of SEMA3E, which binds with high affinity to PLEXIN-D1, the other class 3 SEMAs require an association with NRPs to exploit their activities. NRPs are transmembrane proteins mainly involved in the regulation of the functions of plasma membrane receptors, including plexins, vascular endothelial growth factor receptors (VEGFR), and integrins 4 and 5. Although the mechanisms by which PLEXIN A and B modulate cell functions have yet to be defined, the structural analysis of their cytosolic sequence reveals the presence of a GTPase activating protein (GAP) domain

that is divided into two segments by a Rho GTPase-binding domain, and suggests a general and prominent role of monomeric G proteins (reviewed in 2, 3 and 6).

Two seminal studies provided innovative breakthroughs that suggested a key role of SEMAs in cardiac and also vascular development (Table 1). The first study described the phenotype of *Sema3A*$^{-/-}$ mice, including hypertrophy of the right ventricle and dilation of the right atrium [7]. Later, it was demonstrated that neuropilin-1 (NRP1) was expressed on the surface of endothelial cells (ECs) and bound vascular endothelial growth factor (VEGF)A-165, an isoform containing a heparin-binding domain encoded by exon 7. This interaction enhanced binding to VEGFR2 [8], allowing stabilization of the tertiary complex [9]. The relevance of this mechanism was further reinforced by the analysis of *Nrp1−/−* mice [10] that showed a pattern reminiscent of human congenital heart diseases (CHDs) caused by microdeletions of chromosome*22q11*, including the disorganized development of the branchial arch system, the partial regression of the dorsal aorta (see Glossary), and insufficient septation of the truncus arteriosus.

Table 1.

SEMAs and SEMA receptors involved in cardiovascular medicine

SEMA	Receptor[a]	Normal functions[b]	Pathological functions[b]	Refs
3A	Plexin A1–A4; NRPs, VEGFR2	• Lymphangiogenesis • Vessel patterning	• Arrhythmias • Atherosclerosis • Charge Syndrome • Glomerulonephritis • Heart failure • Tumor angiogenesis • Vascular ischemia	7, 10, 20, 24, 34, 36, 37, 38, 39, 40, 43, 48, 49, 60, 61, 62, 63, 65, 66, 67, 68, 72, 73, 74 and 75
3C	Plexin A2,D1; NRP 1–2	• Regulation of NCC migration	• Conotruncus CHDs	13, 15, 16, 17, 18, 19, 20, 22 and 23
3D	Plexin A1–4; NRP1		• Abnormal pulmonary veins	[25]
3E	Plexin D1, B2; NRP1–2;	• Vessel patterning	• Atherosclerosis • Conotruncus CHDs • Vascular ischemia • Tumor angiogenesis	22, 27, 28, 29, 30, 42, 44, 45, 60, 63, 64 and 69
3F	Plexin A3, 4; NRP 1–2		• Tumor angiogenesis	54, 73 and 76
4A	Plexin B2, D1	• Vessel patterning	• Vascular ischemia	17 and 59
4D	Plexin B1–2, C1; Met		• Atherosclerosis • Tumor angiogenesis	41, 70 and 71
5A	Plexin A3, B3;		• Tumor	[77]
6A	Plexin A1; VEGFR2; Off-track	• Regulation of NCC migration • Vascular patterning	• Tumor angiogenesis	15 and 78
6B	Plexin A1; VEGFR2; Off-track	• Regulation of NCC migration		[15]
6D	Plexin A1; VEGFR2; Off-track	• Development of endocardium and myocardium		[79]

a The indicated co-receptors are involved in vascular systems; other co-receptors are described in other cellular systems [5].

b The data reported here exclusively refer to models of a specific disease, genetic engineered mouse models, or analysis of human samples.

SEMAs and heart development

The formation of the heart consists of a process in which the primitive heart tube is separated into pulmonary and systemic circulations. This process includes the appropriate septation of the cardiac chambers, the atrioventricular or ventriculo-arterial connections, and the differentiation of great vessels. SEMAs and their receptors exert profound effects on these steps, participating in controlling the involvement of the neural crest (NC) cells and progenitors originating from the second heart field. Different mouse models in which the SEMA system has been genetically manipulated clearly indicate that it plays a relevant role from E8.5 to E12.5 of murine embryogenesis. During this period, the heart starts to loop (at E8.5), and by E10.5 it acquires well-defined chambers that are definitively separated at E14.5. The outflow tract (OFT) is formed from E8.5 onwards with the appearance of the aortic sac and truncus arteriosus communis, and is largely remodeled at E12.5 with septation of the aortic and pulmonary channels. At the same time, great vessels experience deep changes leading to the asymmetry that characterizes their final shape.

SEMAs and the formation of the ventricular OFT

The OFT consists of the primitive right ventricle, ventricular septum, and conotruncus, which is fundamental in the remodeling process of the bi-circulation system through differentiation into semilunar valves and the subpulmonary infundibulum. Alterations in the OFT result in cardiac malformations, such as tetralogy of Fallot, pulmonary atresia, double-outlet right ventricle, truncus arteriosus, aortic arch anomalies, and DiGeorge syndrome. Based on animal studies, the morphogenesis of the OFT seems to require the orchestrated activities of some SEMA-mediated pathways.

Global or endothelial-specific ablation of *Nrp1* 10 and 11 results in cardiac defects that are reminiscent of DiGeorge syndrome (Figure 1), which is strictly connected to an impairment of the recruitment of the cardiac neural crest cells (NCCs) and mainly characterized by conotruncus defects. Similarly, the ablation of VEGFA-165 [12], or of the T-box transcription factor 1 (TBX1) [11], which lies within the commonly deleted region of chromosome 22q11, also results in a DiGeorge-like syndrome. However, detailed analysis of these models suggests that different mechanisms are involved. In particular, the absence of NRP1 does not alter NCC migration to the OFT, or the function of the second heart field, which is observed in *Tbx1−/−* mice. Additionally, in VEGFA-165 mutants, cell migration from the NC is normal. However, defects in the fourth pharyngeal arch arteries are more prominent in NRP1 and VEGFA mutants than in*Tbx1−/−* mice. By contrast, *Tie2Cre/Nrp1−/−* mice, which specifically lack NRP1 expression in the endothelium, do not show the atrial myocardium defects seen in VEGFA-165 mutants, suggesting that only some of the features of murine DiGeorge-like syndrome are mediated by the VEGFA/NRP1/VEGFR2 axis, whereas others involve NRP1 as a co-receptor of plexins.

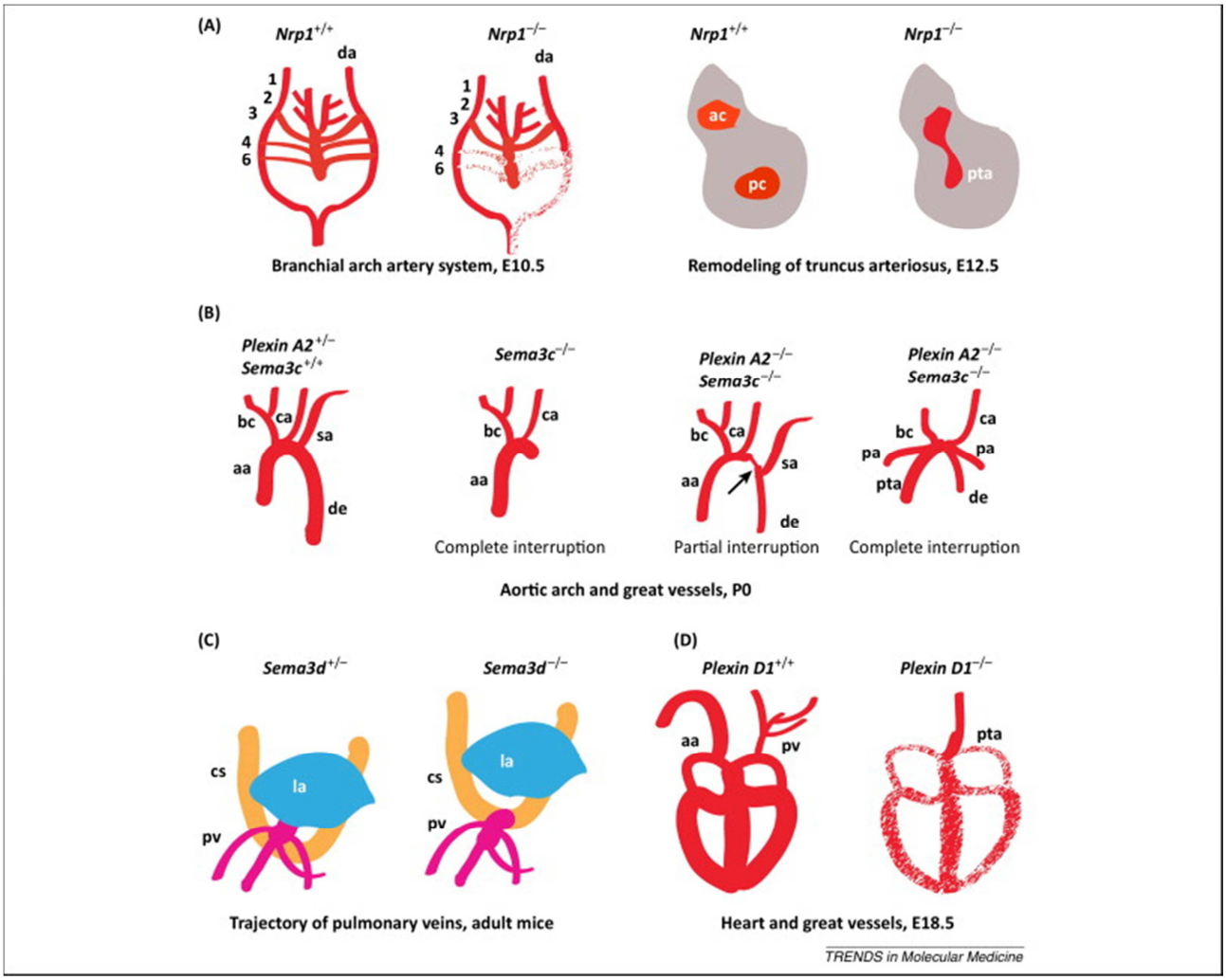

Figure 1.
Schematic representation of the most frequent cardiovascular vascular defects in mouse models of semaphorin (SEMA)/plexin pathways. (A) Insights from neuropilin-1 (*Nrp1*)$^{-/-}$ mice. The great vessels (left) and the aorticopulmonary truncus (right) in embryos are depicted at E10.5 and E12.5, respectively, with branchial arch arteries numbered. The formation of the arch arteries and the dorsal aorta (da) in *Nrp1*$^{-/-}$ mice fails, with the loss of the fourth and sixth arch arteries from both sides, as well as abnormalities of the left dorsal aorta. The truncus is completely separated in *Nrp1*$^{+/+}$ mice, separating the aortic channel (ac) and pulmonary channel (pc). *Nrp*$^{-/-}$ mutants display a partial separation, resulting in a persistent truncus arteriosus (pta). (B) Insights from *Sema3c*$^{-/-}$ and *Plexin A2*$^{-/-}$ mice. Depiction of the aortic arch (aa), descending aorta (de), brachiocephalic artery (bc), left common carotid artery (ca), and left subclavian artery (sa) at P0. The analysis of mutants at P0 shows the incomplete (black arrow) or complete interruption of the aortic arch, with absence of the descending aorta and left subcalvian artery. When the interruption is complete, persistent truncus arteriosus is usually observed. Other defects described in differing percentages are septal defects and duplication of the left common carotid artery (*Sema3c*$^{-/-}$). (C) Insights from *Sema3d*$^{-/-}$ mice. The figure depicts the connection between the pulmonary veins and the heart of newborn mice. Pulmonary veins (pv) connect to the left atrium (la) in wild-type mice, whereas *Sema3d*$^{-/-}$ mutants have an anomalous connection to the coronary sinus (cs), resulting in enlarged atria and ventricles, consistent with a left-to-right flow shunt. (D) Insights from *Plexin D1*$^{-/-}$ mice. Schematic of a normal heart with ascending aorta (aa) and pulmonary veins is shown. At birth, *Plexin D1*$^{-/-}$ mutants show persistent truncus arteriosus (pta), thin mycordium, and abnormal distribution of the coronary vessels.

The analysis of these phenotypes envisages a possible flow of events. TBX1, which does not directly control cardiac NCCs, can impact tissue-specific gene expression, thereby altering the environment through which the NCCs migrate. VEGFA and NRP1 are likely to act later by regulating the circuits between the endothelium and the cells that have migrated from the NC and second heart field 11 and 12. Alternatively, the general vascular defects observed in VEGFA and NRP1 mutants may cause detrimental hypoxic effects in specific steps of cardiogenesis.

Targeted inactivation of SEMA3C causes impaired migration of the NCCs to the developing OFT, resulting in interruption of the aortic arch and persistent truncus arteriosus [13] (Figure 1), which is also observed in ablation of the cardiac NC [14]. Therefore, SEMA3C seems to be involved in the recruitment of cardiac NCCs in the OFT. This model is supported by expression analysis of SEMA3C and its putative receptors. SEMA3C is mainly present in the OFT and subpulmonary myocardium, whereas PLEXIN A2, PLEXIN D1, and NRP1 are present in cardiac NCCs 15 and 16. Accordingly, *Plexin D1−/−* and *Plexin A2−/−* mice15 and 17 exhibit OFT defects (Figure 1) and the ablation of GATA binding factor 6 (GATA6), which regulates the expression of both SEMA3C and PLEXIN A2, phenocopies the persistent truncus arteriosus observed in *Sema3c−/−* mice [18]. Interestingly, patients with mutations in *GATA6* and *PLEXIN D1* have truncus arteriosus defects 18 and 19, and patients with *1q32.2* deletions (the *PLEXIN A2* locus) are affected by tetralogy of Fallot [20]. The function of PLEXIN A2 is further supported by the NCC-specific deletion of *Brg1*, a subunit of the switch/sucrose non-fermentable (Swi/Snf)-like Brg1-associated factors (BAF) chromatin-remodeling complex, which is indispensable to the *Plexin A2* promoter. Such animals show defects in the OFT [21].

The role of PLEXIN D1 is not restricted to the migration of cardiac NCCs. The inactivation of *Plexin D1* in ECs not only causes defects in the OFT, but also in the myocardium and coronary vessels, suggesting that PLEXIN D1 activates the endothelial functions necessary for correct cardiogenesis 22 and 23 (Figure 1).

The attractive effects of SEMA3C are counteracted by the repulsive actions of SEMA6A and SEMA6B [15], and the final result is more efficient navigation of the cardiac NCCs toward the nascent heart. Non-synonymous mutations of *SEMA3A* have been found in Charge syndrome, a complex disorder with OFT defects and abnormal migration of NCCs [24], further suggesting that SEMA3A is involved in the navigation of these cells.

SEMA3D: a pleiotropic effector of heart development

A missense mutation in *SEMA3D* (F602L) with autosomal-dominant inheritance is present in patients with total anomalous pulmonary venous connection [25], in which the connection between the pulmonary veins and the left atrium is absent. Consequently, pulmonary venous blood is delivered to the right side of the heart rather than to the left side. *SEMA3D* F602L shows reduced chemorepulsive activity in ECs, suggesting that this variant may be involved in the pathogenesis of this disorder [25]. *Sema3d−/−*mice have an anomalous connection of the pulmonary veins to the coronary sinus or right atrium, as well as heart enlargement. These defects are connected by modified vascular patterning in the region of the nascent pulmonary veins [25]. During the separation of the primitive venous plexa, which leads to the systemic and pulmonary venous cardiac inflow, SEMA3D is expressed in the mesocardial invagination, which limits the areas of the splanchnic mesoderm and the venous pole of the heart. This distribution allows for the restricted migration of the mid-pharyngeal ECs that form the pulmonary vein. In later stages, SEMA3D rings the path of the solitary pulmonary vein [25]. In the absence of SEMA3D, the pulmonary vasculature stochastically forms anomalous connections to the adjacent venous plexa [25].

The ability of SEMA3D to define the borders of specific vascular areas is shared with SEMA3E, which is another endothelial chemorepulsive molecule [26]. The absence of SEMA3E is characterized by intersomitic vessels with disordered trajectories [27], and by the loss of dorsal aortae, which are substituted by a highly branched plexus [28]. These studies indicate that SEMA3E is instrumental in creating an avascular zone that limits the navigation of specific structures in the vascular tree. In addition to its direct chemorepulsive effect on ECs, SEMA3E upregulates the expression of soluble VEGFR1, which acts as a decoy that deregulates the proper levels of VEGF required for vascular patterning [29]. Despite the dramatic effect of the absence of SEMA3E on the remodeling of trunk vessels, SEMA3E knockout mice are viable, indicating that redundant mechanisms support the final vasculature shaping of the great arteries[30].

Coronary vessel formation is tightly connected to the development of the epicardium, which originates from the proepicardium, a mass of coelomic progenitors located at the venous pole of the embryonic heart. From the proepicardium organ, cells migrate across the heart surface to form the epicardial sheet, which later produces multipotent subepicardial mesenchymal cells via epithelial–mesenchymal transition. The epicardial origin of most coronary smooth muscle cells and fibroblasts is accepted; however, the coronary EC origin is still debated. Experiments performed in avians indicate that the epicardium differentiates into the endothelium, whereas lineage tracing in mice does not support this origin [31]. Recent lineage findings delineate a sub-population of epicardial precursors in mice and chicks that are non-overlapping with the TBX18- and Wilms' tumor 1- (WT-1) expressing populations, and they express

SEMA3D and scleraxis, respectively. Proepicardial cells expressing SEMA3D directly produce the coronary endothelium [32]. Interestingly, derivatives of SEMA3D and scleraxis precursors populate the sinus venous and endocardium, respectively, which have been implicated in giving rise to the coronary endothelium in mice[33]. Therefore, the discovery of a specific SEMA3D-positive cell in the proepicardial organ reconciles the previous contradictory data from chicks and mice.

SEMAs and cardiac innervation

Sympathetic innervation is critical for global cardiac activity, and defects in electrical conduction alter the heart rate. On the basis of SEMA activity on autonomous nervous systems 2 and 3, SEMA3A and NRPs are candidates for regulating cardiac innervation. The hearts of *Sema3a−/−* mice [34] have an altered patterning of sympathetic fibers from the subepicardial to the subendocardial regions without alterations of the conduction system. These defects result in sinus bradycardia. Interestingly, a similar phenotype is observed in mice lacking *Nrp1* in NCCs [35]. The overexpression of *Sema3A* in the adult myocardium reduces the number of sympathetic fibers and increases susceptibility to the arrhythmogenic effect of catecholamines, indicating that SEMA3A is relevant for sympathetic functions, not only during development, but also after birth [34]. The inhibitory effect of SEMA3A on sympathetic innervation has been positively exploited in a model of cardiac ischemia [36]. The expression of SEMA3A in the border zone of the infarcted area reduces sympathetic hyper-innervation, decreasing the prolonged QT intervals and the inducibility of ventricular tachyarrhythmias. These models explain the recent report that unexplained cardiac arrest with documented ventricular fibrillation is characterized by a high incidence of a the single nucleotide polymorphism, resulting in the modified SEMA3A-I334 V [37]. Interestingly, SEMA3A-I334 V shows reduced *in vitro* neural chemorepulsive activity [37].

In addition to the regulation of heart innervation, SEMA3A can directly influence the repolarizing phase of the cardiac action potential. SEMA3A binds a cardiac K^+ voltage gated channel (K_v 4.3) and inhibits the transient outward current mediated by this channel [38]. Four missense mutations in SEMA3A were identified in patients with Brugada syndrome, a congenital disease with conduction abnormalities and an increased risk of ventricular arrhythmias. Two mutants identified in two patients with this syndrome (SEMA3A-R552C and SEMA3A-R734W) showed a reduced activity in blocking the outward current mediated by K_v 4.3 [38].

SEMAs and embryonic angiogenesis

During organogenesis, the primitive vascular plexus undergoes deep remodeling, which results in specific stereotypic vascular patterning. In many anatomical districts, the close association of vessels and nerves reflects the functional interdependent relationship between the two systems. Many experiments suggest that nerves are the first to develop and that tutor vessels form around the neural network. SEMAs can exert attractive or repellent functions on both ECs and axons 2, 3 and 6. These activities are key determinants of the cross-talk between the components of the neurovascular units, and they influence their spatial organization. A consistent vascular feature of mice that lack a member of the SEMA or PLEXIN families is the presence of vessel defects that parallel nerve defects. For example, in *Sema3a−/−* mice, the pattern of peripheral nerve growth in the limb skin is altered, forcing the vasculature to align with the disorganized peripheral nerves [39]. Similarly, silencing either *Sema3a1*, *Sema3a2*, or *Plxnd1* in zebrafish embryos results in a comparably aberrant and unrestrained morphogenesis of intersegmental blood vessels, which are located between each pair of somites [40]. These results have been confirmed in *Sema3a−/−* mice (reviewed in [41]). A similar phenotype is described in both *Plexin-d1−/−* and *Sema-3e−/−* mice, in which the intersomitic vessels are not excluded from the caudal region where SEMA3E is expressed and the vessels extend ectopically through the somites [42].

In the kidney, sympathetic innervation is strictly connected to afferent glomerular arterioles, and *Sema3a−/−*mice examined at birth, when kidney maturation is not completed, show massive defects in renal vascular patterning and increased proliferation of glomerular ECs [43]. As a consequence, many podocytes have wide foot processes and the glomerular filtration barrier is altered. Interestingly, transcription of both*Sema3b* and *Sema3e* was upregulated in these knockout mice [43], indicating that homeostatic transcriptional gene circuits may have evolved to preserve *Sema3* function during vascular development. The liver sinusoid consists of fenestrated ECs that are discontinuously surrounded by stellate cells, which are homologous to pericytes in vascular capillaries. Different nerve ending types are located in Disse's space and face the wall of sinusoids. PLEXIN-B2, the putative receptor of SEMA4A and 4E [6], is expressed in sinusoidal ECs, and PLEXIN-B2 knockout results in profound disorganization of the lobular circulation architecture [17].

A second model of cooperation between the nerves and vasculature is inferred by the organogenesis of the neurovascular unit of whisker follicles and retinas. An internal ring of sensitive nerves and an external ring of capillaries surround the follicles. The complementary and dynamic expression pattern of SEMA3E and PLEXIN-D1 during follicle development suggests their role in the formation of ring structures. SEMA3E delimits the follicle and, taking advantage of its endothelial repulsive activity through the PLEXIN-D1 expressed in blood vessels, delimitates the border of the ring [44]. PLEXIN-D1 has significant expression in axons when the nerve ring is structured, which most likely favors proper navigation. However, PLEXIN-D1 expression disappears in the nerve terminal when the neurovascular structures are definitively organized, enabling the nerve to innervate the areas expressing SEMA3E [44]. A similar mechanism is described in the retinal vasculature [45]. Retinal ganglion cells uniformly express SEMA3E; however, PLEXIN-D1 is only expressed at the front of developing capillaries. This suggests that the dynamic and differential expression of the receptor is crucial for establishing patterned vasculature. In particular, the SEMA3E/PLEXIN-D1 axis negatively regulates the VEGF-induced Delta-like4 (Dll4)-NOTCH signal, which controls the harmonic progression of capillaries from the macula to the periphery of the retina. The ablation of the SEMA3E/PLEXIN-D1 axis results in an irregular growth front and a less-branched network [45]. The vascularization of whisker follicles and the retina suggests that the target organ produces a SEMA member and that the tissue-modulated expression of the cognate receptor is instrumental in determining the final shape of both the vascular and nervous networks.

SEMAs and lymphangiogenesis

Although less evident than the nervous system and blood vasculature, lymphatic vessels parallel nerves46 and 47, suggesting that there are possible interplayed loops. SEMA3A, PLEXIN A1, and NRP1 are all expressed in lymphatic vessels, and the deletion of *Sema3a* and *Plexin A1* results in prominent lymphatic defects 48 and 49. Mutant mice show normal differentiation of lymphatic ECs with reduction in the size of mesenteric lymphatics and increased coverage by smooth muscle cells. Abnormal smooth muscle coverage of the lymphatic valves and the loss of valve leaflets are features of the deletion of *Foxc2* [50], and they contribute to lymphedema distichiasis in patients with *FOXC2* mutations [51], highlighting the importance of the coordinated extension of valve leaflet ECs and repulsion of mural cells from the lymph vessel wall.

NRP2 is expressed in the lymphatic system and its ablation reduces the number of small lymphatic vessels[52]. Additionally, the inhibition of NRP2 blocks VEGF-C/VEGFR3-mediated tumoral lymphangiogenesis[53]. By taking into account the chemorepulsive effect of SEMA3F on lymphatic ECs [54], we may envisage that it can act as an anti-lymphangiogenic molecule. In addition, SEMAs are involved in immune cell trafficking across lymphatic vessels. *Sema3A*, produced by lymphatic ECs, promotes the entry of dendritic cells into the afferent lymph node lymphatics, and the lack of the cognate receptor PLEXIN-A1 results in decreased dendritic cell trafficking to the draining lymph nodes [55].

SEMAs in vascular pathology

Tumor angiogenesis has been the first area showing that SEMAs have a role in vascular mechanisms associated with pathological events. This issue has been extensively investigated and discussed in outstanding and comprehensive reviews 41, 56 and 57. Box 1 discusses an emerging aspect of the biology of class 3 SEMA describing the ability of SEMA3A and 3F to restore the chaotic architecture of tumor vasculature [58].

Box 1.

Class 3 SEMA promotes the normalization of tumor vasculature

The role of SEMAs in tumor angiogenesis has been demonstrated in xenograft models 54 and 80 and reflects the pro- and anti-angiogenic activities of the different molecules of this family (reviewed in56 and 57). However, class 3 SEMAs have promising anti-angiogenic therapeutic potential [73]. A new rationale for the use of anti-angiogenic compounds is to normalize the capillary network in the tumor, which is chaotic, poorly perfused, and favors metastatic diffusion, increased interstitial pressure and reduced bioavailability of drugs (reviewed in [81]).

SEMA3A is expressed by ECs during the angiogenesis of pre-malignant lesions, and is lost during tumor progression when aberrant angiogenesis occurs. The reintroduction of SEMA3A into transgenic mouse models of neuroendocrine pancreatic tumor or uterine cervix carcinoma resulted in reduced vascular density, improved pericyte coverage, inhibition of tumor growth, and survival extension. Remarkably, these modifications are associated with a normalizing effect and rescue from the hypoxic state [73]. Interestingly, the administration of SEMA3A, either alone or in combination with

sunitinib, enhances the amount of doxorubicin delivered to the tumors. Furthermore, the strong anti-angiogenic effect of molecules blocking VEGF pathways activates a hypoxia-mediated metastatic phenotype, which is reversed by the normalizing activity of SEMA3A [74]. SEMA3F has a similar activity. It is down-regulated in Schwannomas and its re-expression in neurofibromatosis-2 null cancer cells efficiently decreases tumor growth and normalizes the vasculature [76].

The precise mechanisms by which class 3 SEMAs normalize blood vessels are unknown. An initial hypothesis focuses on the ability of SEMA3A to promote the chemotaxis of pericytes [73], which are fundamental to the function of the vascular unit. Furthermore, SEMA3A recruits a specific population of $NRP1^{+}$, $Cd11b^{+}$, and $Gr\text{-}1^{-}$ monocytes into tumors in different mouse models of cancer. This population produces several factors that are involved in vessel maturation [including platelet-derived growth factor β (PDGFβ), transforming growth factor β (TGF-β), thrombospondin-1, and chemokine (C-X-C motif) ligand 10 (CXCL10)], as well as halting cancer growth and normalizing tumor vasculature[82].

However, the role of SEMA3A in recruiting regulatory myeloid cells is more composite and possibly reflects different timing of tumorigenesis. For example, NRP1 is expressed on macrophages and enables them to move toward a SEMA3A gradient. This response is lost in hypoxic areas, where NRP1 is downregulated and elicits the entrapment of macrophages in the hypoxic niche. The hypoxic environment favors a macrophage phenotype supporting invasive growth [75].

Ischemic diseases

After an ischemic injury, hypoxia triggers a transcriptional response that includes the attempt to rescue blood flow by overexpressing VEGF-A. However, the final response is often limited and inefficient for recovering physiological function. Emerging evidence supports the notion that SEMAs have a role in ischemia. SEMA4A from infiltrating macrophages rapidly increases in the early phase of reperfusion after myocardial ischemia and stimulates VEGF-A production, contributing to the revascularization of the damaged area [59]. Interestingly, SEMA4A itself is also able to recruit macrophages to the injured areas[59].

SEMA3E and its receptor PLEXIN D1 are also upregulated in limb ischemia of normal or diabetic mice [60], and dampen the pro-angiogenic activity of VEGF-A. The ectopic expression of SEMA3E abolishes the therapeutic effect of the injection of a VEGF-A-encoding vector in injured tissues. Furthermore, the inhibitory effect of SEMA3E is reversed by the expression of the soluble form of PLEXIN D1, which acts as a decoy. The difficult and delayed repair of vascular injuries in diabetes patients could be due to SEMA3E overexpression. A similar scenario occurs in normal skeletal muscles overexpressing both VEGF-A and SEMA3A by adenovirus associated vectors. In this model, SEMA3A blocks the effect of VEGF-A by promoting EC apoptosis and inhibiting the formation of capillaries and arterioles [61].

SEMAs also play roles in ischemia of the nervous system. SEMA3A is upregulated in experimental models of stroke 62 and 63 and ischemic retinopathies 64 and 65. In models of oxygen-induced retinopathy, SEMA3A production by neurons is triggered by the inflammatory cytokine interleukin 1β (IL-1β) released by activated microglia 65 and 66. When neurons produce an angiogenesis inhibitor such as SEMA3A, they quarantine the injured areas by poorly efficient revascularization. It is a realistic hypothesis that the lack of a prompt and adequate vascular supply in neural ischemia, which would otherwise be necessary for recovering the deprived neurons, is compensated by the mounting of a repulsive front that shunts metabolic resources away from the compromised tissues, saving the less injured regions. Furthermore, the presence of the high levels of SEMA3A produced by the ganglion cell layer in ischemic retinas can explain the deregulated VEGF-A-induced extra-retinal vascular outgrowth, which can cause vision-impairing hemorrhage and retinal detachment 64 and 65. SEMA3A blocks angiogenesis in the ischemic area and repels capillaries toward the vitreous. The removal of SEMA3A accelerates the vascular regeneration of neural tissues, improves retinal function, and reduces the risk for intra-vitreal neovascularization. SEMA3A also shows an active role in macular edema, at least in diabetic retinopathy [67]. SEMA3A is increased in the vitreous in the early phase of diabetes in humans and mice. Vascular leakage increases with increasing concentrations of SEMA3A, and the intra-vitreal injection of this molecule increases the permeability of the retinal vasculature. The inhibition of SEMA3A that is achieved by a specific shRNA and by soluble NRP1 (as a trap molecule) prevents the increased permeability of diabetic retinas.

The SEMA3E/PLEXIND1 axis acts similarly to SEMA3A. In the developing retina, this pathway mediates the retraction of endothelial filopodia and balances the VEGF-A-induced migration of tip cells, enabling correct vascular pathfinding [64]. In mouse models of retinopathy, SEMA3E is normally expressed in the ganglion cell layer, but the ECs of the extraretinal

vessels express high levels of PLEXIN D1, which is present in a negligible amount in the intra-retinal vessels. By combining the use of soluble PLEXIN D1, recombinant SEMA3E and genetic approaches, it has been demonstrated that this pathway restores efficient revascularization with sufficient orientation in ischemic retinas [64]. In fact, intra-vitreal SEMA3E injection selectively prevents extra-retinal vascularization without interfering with vascularization of the ischemic retina.

Atherosclerosis

Inflammatory stimuli orchestrate the onset and progression of atherosclerotic plaques. In particular, the EC surface changes and favors the adhesion of circulating leukocytes; monocytes and T cells are recruited to initiate an immune response; smooth muscle cells migrate from the media to the intima; and new and abnormal vessels are generated, facilitating plaque rupture, which in turn cause thrombotic events. The chemorepellent activity of SEMAs is exploited differently in the atherosclerotic process 68 and 69. SEMA3A contributes to the maintenance of the anti-adhesive properties of ECs and halts monocyte diapedesis. In *Ldlr−/−* mice, which develop atherosclerosis when fed on a Western diet, the expression of SEMA3A is severely reduced in the arterial areas that are characterized by a turbulent and pro-atherosclerotic blood flow. *In vitro*, the expression of SEMA3A is reduced by chemokines that are involved in the early phase of atherosclerosis, CCL-2 and IL-8, and by oscillatory flow that mimics the hemodynamic conditions occurring in areas of branching or high curvature, where plaques are usually formed [68] (Figure 2).

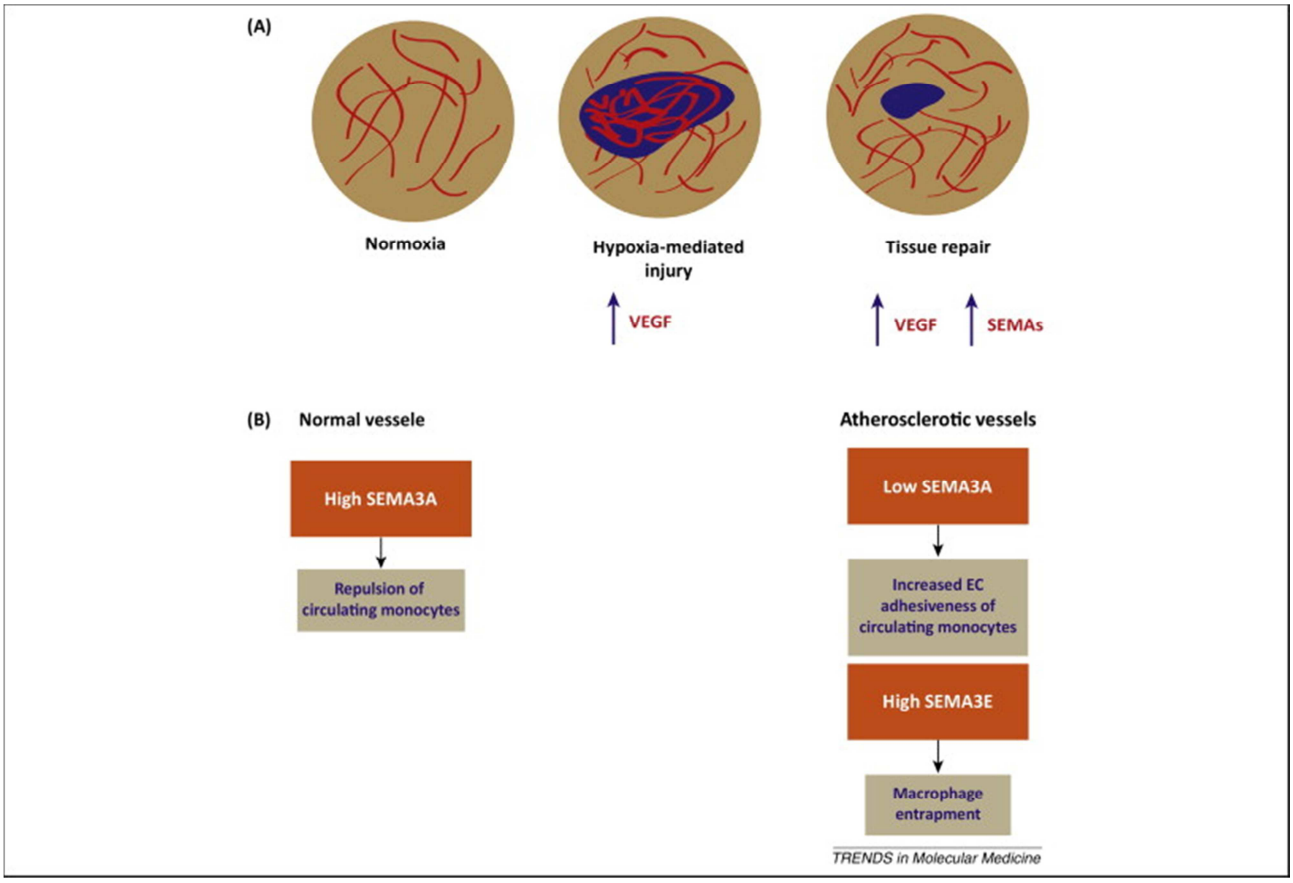

Figure 2.

The role of SEMAs in ischemia and atherosclerosis. (A) In ischemic tissues, hypoxia (purple) mediates a cellular response characterized by an increased expression of VEGF-A, which mediates an angiogenic response. In limb, retina, and brain ischemia, the effect of VEGF is counteracted by the SEMA system (by SEMA3A or SEMA3E). The balance between VEGF-A and SEMAs results in the partial recovery of damage. (B) In normal blood vessels, high SEMA3A expression inhibits the adhesion of circulating monocytes to endothelial cell (EC) surfaces and blocks diapedesis. During atherosclerosis, SEMAs exert chemorepellent and chemoattractive activities. SEMA3A is down-regulated, thus facilitating monocyte recruitment, whereas SEMA3E is expressed by macrophages and inhibits their clearance by inhibiting chemokine-activated motility.

In contrast to SEMA3A, SEMA3E promotes macrophage retention in the plaque and blocks their emigration through the lymphatics, which is observed in acute inflammation. In *ApoE−/−* mice fed a Western diet, M1 pro-inflammatory macrophages located in the atherosclerotic plaque during the progression of the disease exhibit a SEMA3E/PLEXIN-D1 autocrine loop that is repressed when the plaques are regressing. Interestingly, the *in vitro* treatment of macrophages with oxidized lipoprotein simulates the *in vivo*observation. Furthermore, SEMA3E inhibits the macrophage chemotaxis induced by the CCL2 and CCL19 chemokines (Figure 2) [69].
SEMA4D is a transmembrane molecule that becomes soluble after shedding. In addition to its role in the nervous and vascular systems, it has a wide range of activities in immune cells, including the regulation of monocyte and dendritic cell movements and the activation of B cell expansion. The deletion of *Sema4D* in*ApoE−/−* mice delays atherosclerosis [70]. This phenotype is associated with a decrease in the numbers of infiltrating macrophages and capillaries. Human atherosclerotic plaques express high levels of SEMA4D as well as foam cells. Furthermore, SEMA4D inhibits the differentiation of macrophages into foam cells, which is otherwise induced by oxidized lipoproteins by down-modulating the expression of CD36, which has a relevant role in lipoprotein trafficking and atherogenesis [71].

Glomerular diseases
An inducible animal model in which SEMA3A is overexpressed in glomerular podocytes unveils the pathogenic role of this molecule in altering the barrier function of glomerular tuft. Animals develop proteinuria, a capillary injury characterized by swollen ECs and expansion of the basal membrane [72].

Concluding remarks
SEMAs mediate multiple biological activities in the cardiovascular system both during embryogenesis and in adult life. In conjunction with their receptors, NRPs and plexins, SEMAs participate in the development of the heart and remodeling the vasculature during embryogenesis. The identification of specific roles of SEMAs in heart and vascular development is likely to have an important effect on the comprehension of the pathogenesis of some congenital heart and arrhythmogenic disorders. Furthermore, SEMAs and their receptors have crucial roles in maintaining vascular homeostasis in adults and may contribute to the vascular alterations observed in tumors, chronic injuries, and ischemic disorders. Because the network of SEMA signals is rather complex and cell-context-dependent, further studies are required to bring new insights to define the relevance of individual family members in cardiovascular medicine, and to demonstrate a role for SEMAs as diagnostic tools, prognostic predictors and therapeutic targets. Moreover, biotechnological studies and new animal models will aid in the development of therapeutic strategies manipulating SEMA signaling

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Glossary

Angiogenesis
the process describing the transformation of the primitive vascular plexus formed by vasculogenesis into hierarchically organized arteries, capillaries, and veins. This ability is maintained in adult life to sustain the growth of the organs and characterizes several pathological settings. Angiogenesis occurs by sprouting from pre-existing capillaries and requires proteolytic digestion of the extracellular matrix, endothelial cell (EC) migration and proliferation, and their further differentiation in capillaries with a patent lumen. A second mechanism is intussusception. Protrusion of opposing microvascular walls into the capillary lumen creates a bridge between ECs, which represents the template to from a transluminar pillar leading to the formation of two vessels.

Aortic arches
Angioblasts differentiated from the mesoderm of the pharyngeal arches produce six pairs of aortic arches, which are connected to the dorsal aorta and developing cardiac outflow tract (OFT) through the aortic sac. As the caudal vessels become apparent, the most rostral vessels largely disappear and the definitive pattern of the great vessels is gradually established.

Dorsal aortae
the first intra-embryonic blood vessels to arise in the trunk. Primary dorsal aortae comprise a pair of longitudinal vessels in which the anterior ends are connected to the nascent heart via the OFT and the posterior parts are linked to vitelline arteries at the umbilicus level. The left dorsal aorta creates the arch and the descending aorta in adults, whereas the right disappears.

Heart fields
two embryonic areas involved in heart development. The heart develops from a primitive heart tube, which is derived from two primary heart fields located in the lateral plate mesoderm. When this tube undergoes elongation and rightward looping, it represents a scaffold to host the cardiac precursors that are derived from a second heart field consisting of cells located in the pharyngeal mesoderm. These cells contribute to the final shape of the left ventricle, the formation of the OFT, and most of the right ventricle and atrias.

Neural crest (NC)
a multipotent cell population that arises on the dorsal neural tube during development. Neural crest cells (NCCs) delaminate and migrate through the body forming peripheral nervous system and melanocytes. The ectomesenchyme originates from a cranial region of NC and is the source of head and neck tissues. A subpopulation of ectomesenchyle represents the cardiac NC for the importance in different aspects of cardiovascular development. Cardiac NCCs support the development and patterning of the persisting aortic arch arteries into the great arteries and form their smooth muscle layers. They participate in the OFT septation, dividing the common arterial outflow into the aorta and pulmonary trunk.

Finally, NCCs that give rise to parasympathetic innervation of the heart influence the recruitment of precursors from the second heart field to the OFT.

Pulmonary veins

pulmonary veins originate from the midpharyngeal mesenchyme that surrounds the forming lung buds. The first connection of the pulmonary circulation with the heart is mediated by a solitary pulmonary vein, which enters the primary atrium and precedes chamber septation. Subsequent remodeling allows for the final arrangement with four venous orifices

Vasculogenesis

the early formation of the primitive capillary network, which occurs in extra-embryonic and embryonic areas. Angioblasts are the first vascular precursors, which stem from the mesoderm and bear early markers of endothelial lineage. Their commitment and differentiation are regulated by instructive signals from the endoderm. Angioblasts coalesce, form a lumen, and fuse to form a plexus of small blood vessels.