Role of Semaphorin Signaling During Cardiovascular Development

Qianchuang Sun, MD; Shuyan Liu, MD; Kexiang Liu, MD; Kai Jiao, MD, PhD

Semaphorins are a family of secreted and membrane-bound molecules that play critical functions in diverse biological processes. \(^1\) \(^9\) SEMA1A/Fasciclin IV is the founding member of the semaphorin family and was first reported in 1992 as a regulator of axon branching in the growth cone of grasshopper embryos. \(^10\) To date, more than 20 semaphorin members have been discovered in viruses, invertebrates, and vertebrates. \(^3\) \(^5\) \(^11\) In addition to acting as guidance cues for axon growth in the nervous system, semaphorins have been implicated in many other systems, such as immune responses, tumor angiogenesis, bone development and homeostasis, and cardiovascular development. \(^1\) \(^9\)

Semaphorins are categorized into 8 classes. Classes 1 and 2 are found in invertebrates, classes 3 to 7 are present in vertebrates, and class V semaphorins are found only in viruses. \(^3\) \(^12\) All semaphorins contain a conserved domain of about 500 amino acids, termed the sema domain, which is located close to the N-terminal end of the molecule \(^13\) (Figure 1). Crystal structures have revealed that the sema domain is composed of a 7-bladed \(\beta\)-propeller fold, arranged radially around a central axis. Each blade comprises a 4-strand antiparallel \(\beta\)-sheet. \(^14\) \(^15\) With the exception of certain poxvirus semaphorins, other viral and all animal semaphorins contain a plexin-semaphorin-integrin domain in their extracellular regions, immediately to the C-terminal of the sema domain. \(^12\) \(^16\) \(^17\) The plexin-semaphorin-integrin domain is a disulfide-rich motif that is found in plexins, semaphorins, and integrins. In addition to the defined sema and plexin-sema domains, semaphorins can be further distinguished by other specific additional motifs (Figure 1). For instance, classes 2, 3, 4, and 7 contain an immunoglobulin-like domain, class 5 members contain 7 thrombospondin type 1 repeats, and class 3 members contain a basic domain. Some class V members contain an immunoglobulin-like domain, but others do not. \(^3\) \(^12\) \(^16\) Semaphorins can be membrane anchored or secreted. Classes 1, 4, 5, and 6 are transmembrane proteins, whereas classes 2, 3, and V are secreted proteins. Class 7 proteins are glycosylphosphatidylinositol-linked proteins. \(^3\) \(^11\) Some transmembrane semaphorins, such as SEMA6D, also have secreted forms to act both locally and over a long distance. \(^18\) \(^19\)

Plexins are the primary receptors for semaphorins \(^20\) \(^22\) (Table 1). They are segregated into 4 subfamilies, including PLXNA1-4, PLXNB1-3, PLXNC, and PLXND. \(^2\) \(^6\) \(^12\) \(^23\) \(^24\) Similar to semaphorins, all plexins contain a sema domain at their N-terminal ends (Figure 1). The extracellular domains of plexins also contain 3 plexin-sema-integrin motifs and 3 to 6 immunoglobulin-like plexin transcription factors domains. On the intracellular side, plexins are comprised of a rho GTPase-binding domain and a segment GTPase-activating protein domain. \(^2\) \(^6\) \(^12\) \(^23\) \(^24\) Recent structural studies have shown that semaphorins form a homodimer through intermolecular disulfide bridges to trigger plexin signaling. Semaphorins and plexins interact with each other through their respective sema domains in a “head-to-head” orientation. Two plexin molecules bind 1 semaphorin homodimer to form a bivalent 2:2 complex to mediate cell-cell signaling. Proteolytic cleavage of the semaphorin dimer results in its dissociation to monomeric semaphorin, which can still bind plexin but fails to trigger signaling. \(^11\) \(^23\) \(^25\) The same semaphorin molecule can interact with different plexins, and different semaphorins can interact with the same plexins, adding to the complexity of semaphorin-plexin signaling (Table 1). It is noteworthy that the biological activities of the semaphorin-plexin interaction can be further modified by coreceptors. For example, class 3 semaphorins bind to neuropilin (NRP)/plexin complexes, which require NRP1 and/or NRP2 as coreceptors in the complexes. \(^20\) \(^22\) In another example, SEMA6D interacts with the PLXNA4/vascular endothelial growth factor (VEGFR2) receptor complex to stimulate endothelial cell migration in the outflow tract (OFT) region of chicken embryonic hearts.
whereas interaction with the PLEXINA4/PTK7 complex inhibits endothelial cell migration in the ventricle region.18

Although plexins are the best-studied semaphorin receptors, recent findings suggest that other proteins can also act as receptors by binding to the extracellular domain of semaphorins. For example, TIM2, which belongs to the Tim protein family and is characterized by expression on activated T cells and the presence of conserved immunoglobulin domain and mucin domains, is a receptor for SEMA4A.26 CD72 is a novel class of SEMA4D receptor on lymphocytes that belongs to the C-type lectin family.27 Moreover, SEMA7A exerts an essential function by binding and stimulating monocytes through the α1β1 integrin receptor in both the nervous and immune systems.28

Brief Introduction of Cardiovascular Development

Cardiovascular development in embryos can be subdivided into heart development and vascular development. The heart is the first functional organ formed in mammals.9,29-32 Initially, the heart develops from clusters of progenitor cells, which coalesce to form the cardiac crescent and the heart tube.31-33 Subsequently, the heart tube undergoes elongation, looping, septation, remodeling, and maturation to form the final 4-chambered organ.31-33 Multiple cell types, including myocardial, endocardial, epicardial, and neural crest cells (NCCs) act coordinately during complicated cardiogenesis in vertebrates. NCCs are a group of pluripotent cells that are generated at the edge of neural tubes. Cardiac NCCs migrate through pharyngeal arches 3, 4, and 6 to enter the distal region of the OFT, where they play a critical role in separation of the aorta root and pulmonary trunk.34-36 Some cardiac NCCs in the OFT region eventually become interstitial cells in semilunar valves. In addition, NCCs give rise to a group of smooth muscle cells in the pharyngeal arch arteries. In the proximal region of the OFT and atioventricular (AV) canal region, a subset of endocardial cells respond to signals released from the myocardium and undergo epithelial-mesenchymal transition (EMT) to become cushion mesenchymal cells during midgestation. The cellularized OFT and AV cushions facilitate unidirectional blood flow in embryos and are further remodeled into mature septa and valve structures.37-41 Most cushion mesenchymal cells become interstitial cells in valves. Vascular development is initiated with vasculogenesis. In this process, mesoderm-derived angioblasts form a primitive vascular plexus,42 which is further developed into a mature blood vessel system to ensure proper blood supply for the whole body.43

Numerous signaling pathways and downstream transcription factors are required for normal cardiogenesis and blood vessel development.9,31-33,42-51 In the past 2 decades, accumulated studies have indicated that impaired semaphorin signaling results in various cardiovascular disorders during development and in multiple disease states. In this article we summarize recent discoveries regarding the function of semaphorins during mammalian cardiovascular development, with a primary focus on members of the SEMA3, SEMA5, and
SEMA6 families, whose activities during cardiovascular development have been supported with gene inactivation studies in mice.

Versatile Activities of Semaphorins During Cardiovascular Development

Role of Class 3 Semaphorins During Cardiovascular Development

**SEMA3A Role**

SEMA3A regulates cardiac innervation patterning and is essential for heart rate control.52 **Sema3A**−/− mice lack a cardiac sympathetic innervation gradient, which leads to sinus bradycardia, abrupt sinus slowing, and stellate ganglia defects.52 In support of the direct role of SEMA3A in regulating innervation in cardiomyocytes, myocardial-specific overexpression of **Sema3A** resulted in prolonged action potential duration, reduction of sympathetic innervation, and spontaneous ventricular arrhythmia.52 The role of SEMA3A in regulating sympathetic innervation is not limited to developing hearts. In a rat myocardial infarction model, overexpression of **Sema3A** in left stellate ganglion and myocardium reduces sympathetic reinnervation in the myocardial infarction border zone and the susceptibility to malignant arrhythmia.53,54 In an independent gene inactivation study, the major cardiac phenotype of **Sema3A** homozygous mutant mice is postnatal right ventricle hypertrophy.55 The differential phenotypes reported in the literature are likely due to the specific knockout strategies and/or mouse strains used by different groups.

In support of the clinical relevance of SEMA3A function in rodent cardiomyocytes, addition of **Sema3A** to cardiomyocytes derived from human-induced pluripotent stem cells inhibited the Kv4.3 (Ito) channel, as observed in heterologous human embryonic kidney cells.56 Furthermore, several missense mutations in **SEMA3A** (R552C, R734W, and I334V) were shown to be associated with Brugada syndrome and unexplained cardiac arrest.56,57 These mutations impaired the ability of **SEMA3A** to inhibit the **Kv4.3** (Ito) channel.56

**SEMA3C Roles**

**SEMA3C/PLXNA2** signaling and **SEMA3C/NRP1** signaling are required for NCC development, which is essential for proper septation of the cardiac OFT.61-63 Using NCCs isolated from Hamburger Hamilton 10 chicken embryos, Toyofuku et al found that **SEMA3C** promoted NCC migration through **PLXND1** and **NRP1**.64 **SEMA3C** complete knockout mice are cyanotic and die shortly after birth from interruption of the aortic arch, persistent truncus arteriosus, and septation defects in the OFT.61 These morphological defects are likely caused by failure of NCCs to migrate into the proximal OFT.61 A recent study using a conditional gene inactivation approach indicated that **SEMA3C** expressed in NCCs activates **NRP1** in endocardial cells of the OFT to promote EMT in OFT.

---

**Table 1. Semaphorins and Their Corresponding Plexin Receptors**

| Semaphorins | Organisms | Plexins |
|-------------|-----------|---------|
| SEMA1A      | Invertebrate | PLXN A  |
| SEMA1B      | Invertebrate | PLXN A  |
| SEMA2B      | Invertebrate | PLXN B  |
| SEMA3A      | Vertebrate  | PLXN A1, A2, A3, A4, D1 |
| SEMA3B      | Vertebrate  | PLXN A1, A2, A3, A4 |
| SEMA3C      | Vertebrate  | PLXN A1, A2, B1, D1 |
| SEMA3D      | Vertebrate  | PLXN D1  |
| SEMA3E      | Vertebrate  | PLXN B2, D1 |
| SEMA3F      | Vertebrate  | PLXN A1, A3, A4 |
| SEMA4A      | Vertebrate  | PLXN B1, B2, B3, D1 |
| SEMA4B      | Vertebrate  | PLXN B2  |
| SEMA4C      | Vertebrate  | PLXN B2  |
| SEMA4D      | Vertebrate  | PLXN B1, B2, C1 |
| SEMA4G      | Vertebrate  | PLXN B2  |
| SEMA5A      | Vertebrate  | PLXN A1, A3, B3 |
| SEMA5B      | Vertebrate  | PLXN A1, A3 |
| SEMA6A      | Vertebrate  | PLXN A2, A4 |
| SEMA6B      | Vertebrate  | PLXN A1, A2, A4 |
| SEMA6C      | Vertebrate  | PLXN A1  |
| SEMA6D      | Vertebrate  | PLXN A1, D1 |
| SEMA7A      | Vertebrate  | PLXN C1  |
| Poxvirus A39R | Virus     | PLXN C1  |

DOI: 10.1161/JAHA.118.008853

Journal of the American Heart Association
Semaphorins in the Cardiovascular System  Sun et al

Semaphorins are a family of proteins that play crucial roles in cellular migration and vascular development. In the cardiovascular system, semaphorins, such as Sema3D, have been extensively studied for their functions in various developmental processes. For instance, Sema3D is essential for normal septation and pharyngeal arch development.

**SEMA3D Roles**

Functions of Sema3D during cardiovascular development have been found in multiple vertebrates. Knocking down expression of sema3D in zebrafish led to dysmorphic hearts with smaller ventricles, smaller atrium, and thickened myocardial wall.67 Endocardium was present in sema3D-knockdown fish; however, AV valves and trabeculation were absent.

The function of Sema3D in mammals appears to be different from that in zebrafish. Rather, mammalian Sema3D provides repulsive cues to direct normal endothelial cell development. In vitro analyses using primary human umbilical vascular endothelial cells showed that exogenous Sema3D inhibits endothelial cell migration and tube formation and that this activity requires PLXND1/NRP168,69 and activation of the PI3K/AKT pathway.68 Inactivation of Sema3D in mice led to total anomalous pulmonary venous connection in which pulmonary veins abnormally enter the coronary sinus.70 These results suggest that signals provided by Sema3D are particularly important for endothelial cells of pulmonary veins in vivo. Sema3D mice can survive to adulthood but show severe cardiomegaly due to dilation of right atria and ventricles accompanied by left-to-right shunt, which is likely secondary to the total anomalous pulmonary venous connection defect.70 Furthermore, a point mutation (F602L) in Sema3D was identified in a human patient with partial anomalous pulmonary venous connection.70

In addition to the loss-of-function allele, a gain-of-function allele was also identified in a human patient who carried a duplication of the 5’ half of Sema3D.71 This patient displayed transposition of the great arteries, ventricular septal defect, and coarctation of the aorta. The authors speculated that migration of cardiac NCCs into the OFT is impaired in patients. However, a role of Sema3D in NCCs has not been demonstrated using animal models.

**SEMA3E Role**

Sema3E is a potent repulsive guidance cue for endothelial cells. Sema3E mRNA is robustly expressed in the caudal region of each somite in E11.5 mouse embryos from in situ hybridization analysis.72 Knocking out Sema3E led to disorganized intersomitic vessels, suggesting the essential role of Sema3E in guiding intersomitic vessel formation and patterning. Further detailed examination of the Sema3E-null mice revealed more vascular defects, including the paired dorsal aortas and fusion of a large plexus of blood vessels.73,74 To further support Sema3E as a repulsive cue for endothelial cells, electroporation of a Sema3E expression plasmid into E3 chicken embryos reduced vessel formation in the area where Sema3E was ectopically expressed.72

Sema3E appears to act primarily through PLXND1 in blood vessels. Alkaline phosphatase-tagged Sema3E (AP-Sema3E) bound to COS cells expressing PLXND1, but not NRP1 or NRP2.72 AP-Sema3E bound to the blood vessels in sections from wild type but not Plxnd1 null embryos. Functional analysis showed that addition of Sema3E caused collapse of PLXND1-expressing COS cells. Unlike Sema3D, Sema3E-mediated cytoskeletal reorganization does not require NRP1.68 Inactivation of Plxnd1 results in similar organizational defects in the somatic vasculature as observed in Sema3E-null embryos.72

**The Role of Sema5A During Cardiovascular Development**

Sema5A is the only known member of the Sema5 family for which there is clear genetic evidence to support its role during cardiovascular development. Sema5A is a proangiogenic molecule that potently induces endothelial cell proliferation and migration and inhibits apoptosis.75,76 Treatment of immortalized human dermal microvascular endothelial cells with Sema5A significantly increased their migration. Furthermore, subcutaneous injection of Sema5A-containing matrigel enhanced blood vessel sprouting.72 Knocking out Sema5A in mice led to embryonic lethality between E11.5 and E12.5.77 A thorough examination of the cardiovascular system in mutants revealed that the number of secondary and tertiary branches of blood vessels in the cranial region was reduced, although the capillary network was not affected.77 No other cardiovascular defect was reported in mutant embryos. Therefore, the proangiogenic activity of Sema5A is essential only in the cranial region in vivo. The major cause for the death of Sema5A-null embryos remains unidentified, as the vascular defect in the cranial region is unlikely lethal to the embryo.
The Role of Class 6 Semaphorins During Cardiovascular Development

SEMA6A Role

In vitro analysis using human umbilical vascular endothelial cells showed that SEMA6A promotes endothelial cell survival and growth by modulating VEGFR2 signaling.78 The vascular defects in Sema6A-null animals are limited to the eye. At the P4 stage, hyaloid vessels displayed a significantly reduced network complexity in mutant animals. Also at the same stage, the extension of the vascular network from the optic nerve to the periphery was also reduced in mutants. The vascular defects in eyes disappeared at the P8 stage.78 One likely reason is that other members of the semaphorin family are able to compensate for the loss of SEMA6A in mutant eyes at later stages. Sema3A is expressed in the vasculature of eyes during both embryonic and postnatal eye development79 and thus is a likely candidate.

SEMA6A inhibits migration of NCCs isolated from Hamburger Hamilton 10 chicken embryos, in contrast to Sema3C, which stimulates NCC migration.64 However, no defect was observed in the OFT and pharyngeal arch arteries in Sema6A-null mice. It is thus possible that SEMA6A exerts different activities in chicken and mammals.

SEMA6D Roles

The initial evidence indicating a role for SEMA6D during heart development came from chicken studies. It was found that SEMA6D could promote endocardial cell migration in the OFT region through the PLXNA1-VEGFR2 receptor complex, whereas it inhibited endocardial cell migration in the ventricle region through the PLXNA1-PTK7 receptor complex.18 In addition to acting on endocardial cells, SEMA6D can also regulate myocardial wall morphogenesis in chicken embryos through its cytoplasmic domain-dependent reverse signaling.19 The chicken studies described above applied comprehensive loss-of-function and gain-of-function approaches; however, these activities of SEMA6D in chicken were not replicated in subsequent mouse studies.

We recently developed a novel conditional immortal AV cushion mesenchymal cell line, tsA58-AVM, and used this line to identify Sema6D as the regulatory target of bone morphogenetic protein signaling in AV cushions.80 Conditional inactivation of Sema6D in endocardial cells of mouse embryos using the Nfatc1-Cre driver led to hypocellular AV cushions at E9.25 and E9.5 due to reduced EMT in the AV canal region. Functional tests revealed that SEMA6D activates Rho through PLXNA1-FARP1 to promote cushion mesenchymal cell formation in the AV canal80 (Figure 2). Thus, EMT by endocardial cells in the OFT and AV canal both rely on semaphorin signaling, with the OFT region needing Sema3C and the AV canal region requiring SEMA6D. The AV cushion defect in Nfatc1-Cre/Sema6D<sup>loxp/loxp</sup> embryos was resolved at a later stage (E10.5), likely due to the compensatory effect from increased expression of Sema6C.81 Studies of double-knockout mice of Sema6D and Sema6C are required to test this hypothesis. No defect in the OFT cushions or in the endocardial cells of ventricles was observed in Nfatc1-Cre/Sema6D<sup>loxp/loxp</sup> embryos.81 No myocardial wall defect was reported in Sema6D complete knockout mice.81 The apparent difference in the functions of SEMA6D between chicken studies and mouse studies suggests that this cytokine has differential roles in the cardiovascular system in birds and mammals.

Summary

Multiple semaphorin molecules play an essential role in regulating cardiovascular development. This conclusion is supported by convincing mouse genetic evidence complemented with tests on other model systems including cell culture, zebrafish, and chicken studies (summarized in Table 2). The activities of semaphorin signaling are highly versatile and include regulation of NCC migration, endocardial cell EMT in the OFT and AV canal regions, cardiac innervation, myocardial wall morphogenesis, endothelial cell migration during blood vessel formation, and patterning of vessel networks.

There remain some outstanding questions regarding the underlying mechanisms by which semaphorin signaling regulates cardiovascular development. We list 3 here. (1) The functions of semaphorins are highly cell-type and/or tissue-type dependent. In most cases it is unclear how such specificity is achieved. Detailed characterization of conditional gene-inactivation mouse models, in which semaphorin/plexin
genes are specifically inactivated in different cardiovascular cell types, would provide crucial clues to answer this question. 

(2) Semaphorins can activate many downstream effectors to accomplish their complex biological activities. Unlike many other signaling pathways, such as TGFβ/BMP signaling, there is no canonical pathway that is associated with semaphorin signaling. How the semaphorin/plexin complex on the cell surface selectively activates downstream cytoplasmic effectors in a context-dependent fashion remains largely elusive. 

(3) Another critical question to be addressed is how semaphorin signaling interacts with other signaling pathways during cardiovascular development. Such interaction may occur at the cell surface through sharing (or competing for) the same coreceptors and/or in the cytoplasm through crosstalk between different cytoplasmic effectors. 

Answering the above questions will help us design effective strategies to accurately and specifically modulate semaphorin signaling for therapeutic purposes. Recent studies have shown that tumor angiogenesis can be regulated by different semaphorins.\(^4,82\) A better understanding of the molecular mechanism by which semaphorins regulate blood vessel formation may lead to identification of novel intervention targets for cancer treatment. Another potential area for translational research of semaphorin signaling is regenerative medicine. For example, semaphorin signaling is involved in generation of cushion mesenchymal cells in both the OFT and AV canal regions. These mesenchymal cells are precursors of septa and valves in mature hearts. Our knowledge of semaphorin signaling during OFT and AV cushion development may provide crucial guidance for us to differentiate pluripotent stem cells into valvular/septal cells for tissue repair. This area of research remains a blank in the literature. 

In summary, semaphorins are versatile signaling molecules that regulate multiple aspects of cardiovascular development. Studies on semaphorin signaling are highly significant for both basic and translational research.

Acknowledgments

We regret that due to space limitations, the work of all of our colleagues could not be cited here. We thank the members of the Jiao laboratory for their comments and suggestions for the article.

Sources of Funding

Research in the authors’ laboratory is supported by NIH R01 (R01HL095783), R03 (R03HD082634), and R21 (R21CA199586) grants awarded to Jiao.

Disclosures

None.

References

1. Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. Science. 1996;274:1123–1133.

Table 2. Summary of Functions of Different Semaphorins During Cardiovascular Development

| Semaphorins | Cardiovascular Expression | Cardiovascular Defects in Mutants |
|-------------|---------------------------|----------------------------------|
| SEMA3A      | Trabecular zone of embryonic hearts, Purkinje fiber, vascular endothelia cells\(^52,58\) | Zebrafish: Abnormal dorsal aorta development\(^59\) Mouse: sinus bradycardia, abrupt sinus slowing, stellate ganglia defects, right ventricle hypertrophy, abnormal patterning of anterior cardinal veins and intersomitic vessels\(^52,55,58\) Human: Brugada syndrome\(^56,57\) |
| SEMA3C      | Neural crest cells, outflow tract myocardial cells\(^61-63,65\) | Mouse: interruption of the aortic arch, persistent truncus arteriosus, septation defects in the outflow tract\(^61-63\) |
| SEMA3D      | Mesocardial reflection and proepicardial organ in embryos, neural crest cells\(^70\) | Zebrafish: Dysmorphic hearts, absence of atrioventricular valves and trabeculation\(^67\) Mouse: total anomalous pulmonary venous connection, cardiomegaly\(^70\) Human: partial anomalous pulmonary venous connection, transposition of the great arteries, ventricular septal defect, coarctation of the aorta\(^70,71\) |
| SEMA3E      | Notochord, lateral plate mesoderm, caudal region of somites\(^72,73\) | Mouse: disorganized intersomitic vessels, paired dorsal aortas, fusion of a large plexus of blood vessels\(^72-74\) |
| SEMA5A      | Atrium septum, endocardial cells, cushion mesenchymal cells, mesoderm surrounding cranial vessels\(^77\) | Mouse: reduced number of secondary and tertiary branches of blood vessels in the cranial region\(^77\) |
| SEMA6A      | Hyaloid vessels, retinal vessels\(^78\) | Mouse: reduced network complexity in hyaloid and retinal vessels at P4, defects resolved at P8\(^78\) |
| SEMA6D      | Myocardial, endocardial, cushion mesenchymal cells\(^18,19,80\) | Chicken: altered endocardial cell migration, reduced myocardial wall trabeculation, small ventricle\(^18,19\) Mouse: reduced cushion mesenchymal cell number at E9.5, defect resolved at later stages\(^80\) |
Semaphorins in the Cardiovascular System

2. Tamagnone L, Artigiani S, Chen H, He Z, Ming G, Song H, Chedotal A, Winberg MB, Goodman CS, Potten S, Tassier-Lavigne M, Comoglio PM. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell*. 1999;99:71–80.

3. Goodman C, Kolodkin A, Luo Y, Puschel A, Raper J. Unified nomenclature for the semaphorins/collapsins. *Cell*. 1999;97:551–552.

4. Neufeld G, Mumblat Y, Smolkin T, Toledoano S, Nir-Zvi I, Ziv K, Kessler O. The semaphorins and their receptors as modulators of tumor progression. *Drug Resist Updat*. 2016;24:1–12.

5. Gurara S, Tamagnone L. Transmembrane semaphorins: multimodal signaling cues in development and cancer. *Cell Adh Migr*. 2016;10:675–691.

6. Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Yabuki M, Harada K, Hori M. Semaphorins in the cardiovascular system. *Am J Med Genet A*. 2016;171:125–135.

7. Suzuki K, Kumanogoh A, Kikutani H. Semaphorins and their receptors in immune cell interactions. *ImmunoL*. 2008;9:17–23.

8. Kang S, Kumanogoh A. Semaphorins in bone development, homeostasis, and disease. *Semin Cell Dev Biol*. 2013;24:163–171.

9. Epstein JA, Aghajanian H, Singh MK. Semaphorin signaling in cardiovascular development. *Cell Mol*. 2015;21:163–173.

10. Kolodkin AL, Matthes DJ, O’Connor TP, Patel NH, Admon A, Bentley D, Goodman CS. Fasciclin IV: sequence, expression, and function during growth cone guidance in the grasshopper embryo. *Neuron*. 1992;9:831–845.

11. Siebold C, Jones EY. Structural insights into semaphorins and their receptors. *Semin Cell Dev Biol*. 2013;24:139–145.

12. Yazdani U, Terman JR. The semaphorins. *Genome Biol*. 2006;7:211.

13. Kolodkin AL, Matthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. *Cell*. 1993;75:1389–1399.

14. Love CA, Harlos K, Mavaddat N, Davis SJ, Stuart DJ, Jones EY, Esnouf RM. The ligand-binding face of the semaphorins revealed by the high-resolution crystal structure of SEMA4D. *Nat Struct Mol Biol*. 2003;10:843–848.

15. Antipenko A, Himpen J-P, van Leyen K, Nardi-Dei V, Lesnai J, Barton WA, Rajashankar KR, Lu M, Hoemme C, Puschel AW. Structure of the semaphorin-3A receptor binding module. *Neuron*. 2003;39:589–598.

16. Bork P, Doerks T, Springer TA, Snel B. Domains in plexins: links to integrins and transcription factors. *Trends Biochem Sci*. 1999;24:261–263.

17. Gherardi E, Love CA, Esnouf RM, Jones EY. The sema domain. *Curr Opin Struct Biol*. 2004;14:669–678.

18. Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Suto F, Kamei J, Aoki K, Yabuki M, Hori M, Fujisawa H, Kikutani H. Dual roles of Sema6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. *Genes Dev*. 2004;18:435–447.

19. Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Yabuki M, Harada K, Hori M, Kikutani H. Guidance of myocardial patterning in cardiac development by Sema6D reverse signalling. *Cell Biol Cell. 2004;6:1204–1211.

20. Negishi M, Oinuma I, Katoh H. Plexins: axon guidance and signal transduction. *Cell Mol Life Sci*. 2005;62:1363–1371.

21. Fujisawa H. Discovery of semaphorin receptors, neuropilin and plexin, and their functions in neural development. *J Neurobiol*. 2004;59:24–33.

22. He Z, Tessier-Lavigne M. Neuropilin is a receptor for the axonal chemorepellent semaphorin III. *Cell*. 1997;90:739–751.

23. Nogi T, Yasui N, Mihara E, Matsunaga Y, Noda M, Yamashita N, Toyofuku T, Uchida J, Yasui T, Matsumoto M, Yoshida K, Yakura H, Pan C, Parades JR, Kikutani H. Dual roles of Sema6D in cardiac morphogenesis. *Cell Biol Cell. 2004;6:1204–1211.*

24. Hota PK, Buck M. Plexin structures are coming: opportunities for multilevel investigations of semaphorin guidance receptors, their cell signaling mechanisms, and functions. *Cell Mol Life Sci*. 2012;69:3765–3805.

25. Klostermann A, Lohrum M, Adams RH, Puschel AW. The chemorepulsive activity of the axonal guidance signal semaphorin D requires dimerization. *J Biol Chem*. 1998;273:7326–7331.

26. Kumanogoh A, Kikutani H. Semaphorins and their receptors in neural crest cells and cardiovascular development. *J Mol Biol*. 2004;335:598–616.

27. Lin CJ, Lin CY, Chen CH, Zhou B, Chang CP. Partitioning the heart: mechanisms of cardiac septation and valve development. *Develop. 2012;139:3277–3299.*

28. Markwald RR, Norris RA, Moreno-Rodriguez R, Levine RA. Developmental basis of adult cardiovascular diseases: valvular heart diseases. *Ann N Y Acad Sci*. 2010;1188:177–183.

29. Person AD, Klewer SE, Runyan RB. Cell biology of cardiac cushion development. *Int Rev Cytol*. 2005;243:287–335.

30. Hinton RB, Yutzey KE. Heart valve structure and function in development and disease. *Annu Rev Physiol*. 2011;73:29–46.

31. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol*. 1995;11:73–91.

32. Risau W. Mechanisms of angiogenesis. *Nature*. 1997;386:671.

33. Harvey RP. Patterning the vertebrate heart. *Nat Rev Genet*. 2002;3:544.

34. Qian Y, Xiao D, Guo X, Chen H, Hao L, Ma X, Huang G, Ma D, Wang H. Multiple gene variations contributed to congenital heart disease via GATA family transcriptional regulation. *J Transl Med*. 2017;15:69.

35. England G, Granados-Riveron J, Polo-Parada L, Kuriakose D, Moore C, Brook JD, Rutland CS, Satchell K, Gell C, Ghosh TK. Tropomyosin 1: multiple roles in the developing heart and in the formation of congenital heart defects. *J Mol Cell Cardiol*. 2017;106:1–13.

36. Wilting J, Christ B. Embryonic angiogenesis: a review. *Naturwissenschaften*. 1996;83:153–164.

37. Drake CJ, Flemington PA. Vasculogenesis in the day 6.5 to 9.5 mouse embryo. *Blood*. 2000;95:1671–1679.

38. Johnston CM, Capla JM, Grasso CR, Geradini DJ, Callaghan MJ, Kleinman ME, Gurtner GC. Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood*. 2005;105:1068–1077.

39. Chetty SC, Rost MS, Enriquez JR, Schumacher JA, Baltrunaite K, Rossi A, Stainer DY, Sumanas S. VEGF signaling promotes vascular endothelial differentiation by modulating ETβ2 expression. *Dev Biol*. 2017;424:147–161.

40. Baltrunaite K, Craig MP, Desai SP, Chaturvedi P, Pandey RN, Hegde RS, Sumanas S. ETβ2 transcription factors commit Flk1+ cells for tissue regeneration for tumors or angiogenesis. *Angiogenesis*. 2017;20:307–323.

41. Ieda M, Kanazawa H, Kinuma K, Hattori F, Ieda Y, Taniguchi M, Lee J-K, Matsumura K, Tomita Y, Miyoshi S. Sema3a maintains normal heart rhythm through sympathetic innervation patterning. *Nat Med*. 2007;13:604–612.

42. 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;17:608–616.

43. Yang L-C, Zhang P-P, Chen X-M, Li C-Y, Sun J, Hou J-W, Chen R-H, Wang Y-P, Li Y-G. Semaphorin 3a transfection into the left stellate ganglion reduces vulnerability of ventricular tachycardia post-myocardial infarction in rats. *Eur J Med Res*. 2015;18:1866–1896.

44. 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.
Semaphorins in the Cardiovascular System

Sun et al

DOI: 10.1161/JAHA.118.008853

Journal of the American Heart Association

56. Boczek NJ, Ye D, Johnson EK, Wang W, Crotti L, Tester DJ, Dagradi F, Mizusawa Y, Torchio M, Alders M. Characterization of SEMA3A-encoded semaphorin as a naturally occurring Kv4.3 protein inhibitor and its contribution to Brugada syndrome. Circ Res. 2014;115:460–469. DOI: 10.1161/CIRCRESAHA.115.303657.

57. Nakano Y, Chayama K, Ochi H, Toshishige M, Hayashida Y, Miki D, Hayes CN, Suzuki H, Tokuyama T, Oda N. A nonsynonymous polymorphism in semaphorin 3A as a risk factor for human unexplained cardiac arrest with documented ventricular fibrillation. PLoS Genet. 2013;9,e1003364.

58. Serini G, Valdembri S, Morterra G, Burkhardt C, Caccavari F, Zammataro L, Primo L, Tamagnone L, Logan M. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature. 2003;424:391–397.

59. Shoji W, Isogai S, Sato-Maeda M, Obinata M, Kuzawa YJ. Semaphorin3A1 regulates angioblast migration and vascular development in zebrafish embryos. Development. 2003;130:3227–3236.

60. Acevedo LM, Barillas S, Weis SM, G€ustert JR, Cheres PJ. Semaphorin 3A suppresses VEGF-mediated angiogenesis yet acts as a vascular permeability factor. Blood. 2008;111:2674–2680.

61. Feiner L, Webber AL, Brown CB, Lu M-M, Li J, Ma X, Webber AL, Jia L, Feinstein P, Mombaerts P, Epstein JA. PlexinA2 and semaphorin signaling during cardiac neural crest development. Development. 2001;128:3061–3070.

62. Brown CB, Feiner L, Lu M-M, Li J, Ma X, Webber AL, Jia L, Raper JA, Epstein JA. PlexinA2 and semaphorin signaling during cardiac neural crest development. Development. 2001;128:3071–3080.

63. 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.

64. Toyofuku T, Yoshida J, Sugimoto T, Yamamoto M, Makino N, Takamatsu H, Takegahara N, Sato F, Hori M, Fujisawa H. Repulsive and attractive semaphorins cooperate to direct the navigation of cardiac neural crest cells. Dev Biol. 2008;321:251–262.

65. Kodo K, Shibata S, Miyagawa-Tomita S, Ong SG, Takahashi H, Kume T, Okano H, Matsuoka R, Yamagishi H. Regulation of Sema3c and the interaction between cardiac neural crest and second heart field during outflow tract development. Sci Rep. 2017;7:6771.

66. Yang WI, Hu J, Umura A, Tetzlaff F, Augustin HG, Fischer A. Semaphorin-3C signals through Neurip1- and PlexinD1 receptors to inhibit pathological angiogenesis. EMBO Mol Med. 2015;7:1267–1284.

67. Sato M, Tsai H-J, Yost HJ. Semaphorin3D regulates invasion of cardiac neural crest cells into the primary heart field. Dev Biol. 2006;298:12–21.

68. 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;289:17971–17979.

69. Hamm MJ, Kirchmaier BC, Herzog W. Sema3D controls collective endothelial cell migration by distinct mechanisms via Nrp1 and PlexinD1. J Cell Biol. 2016;215:415–430.

70. Degenhardt K, Singh MK, Aghajanian H, Massera D, Wang Q, Li J, Li L, Choi C, Yzaguirre AD, Franey LJ. Semaphorin 3d signaling defects are associated with anomalous pulmonary venous connections. Nat Med. 2013;19:760–765.

71. Sanchez-Castro M, Pichon O, Briand A, Poulin D, Gournay V, David A, Caignec CL. Disruption of the Sema3D gene in a patient with congenital heart defects. Hum Mutat. 2015;36:30–33.

72. 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;307:265–268.

73. Meadows SM, Fletcher PJ, Moran C, Xu K, Neufeld G, Chauvet S, Mann F, Krieg PA, Olaver J, Cleaver O. Integration of repulsive guidance cues generates avascular zones that shape mammalian blood vessels. Circ Res. 2012;110:34–46.

74. Meadows SM, Ratliff LA, Singh MK, Epstein JA, Cleaver O. Resolution of defective dorsal aorta patterning in Sema3E-deficient mice occurs via angiogenic remodeling. Dev Dyn. 2013;242:580–590.

75. Sadanandam A, Rosenbaug EH, Singh S, Varney M, Singh RK. Semaphorin 5A promotes angiogenesis by increasing endothelial cell proliferation, migration, and decreasing apoptosis. Microvasc Res. 2010;79:1–9.

76. Sadanandam A, Sidhu S, Wullschl¥ger S, Singh S, Varney M, Yang C, Ashour A, Batra SK, Singh R. Secreted semaphorin 5A suppresses pancreatic tumour burden but increased metastasis and endothelial cell proliferation. Br J Cancer. 2012;107:551.

77. Fiore R, Rahim B, Christoffels VM, Moorman AF, Puschel AW. Inactivation of the Sema5a gene results in embryonic lethality and defective remodeling of the cranial vascular system. Mol Cell Biol. 2005;25:2310–2319.

78. Segarra M, Ohnuki H, Maric D, Salvadori O, Hou X, Kumar A, Li X, Tosato G. Semaphorin 6A regulates angiogenesis by modulating VEGF signaling. Blood. 2012;120:4104–4115.

79. Ko JA, Mizuno Y, Yanai R, Chikama T, Sonoda KH. Expression of semaphorin 3A and its receptors during mouse corneal development. Biochem Biophys Res Commun. 2010;403:305–309.

80. Peng Y, Song L, Li D, Kesterson RA, Wang J, Wang L, Rokosh G, Wu B, Wang Q, Jiao K. Sema6D acts downstream of bone morphogenetic protein signalling to promote atrioventricular cushion development in mice. Cardiovasc Res. 2016;112:532–542.

81. Takamatsu H, Takegahara N, Nakagawa Y, Tomura M, Taniguchi M, Krieg PA, Cleaver O. Integration of repulsive guidance cues generates avascular zones that shape mammalian blood vessels. Circ Res. 2012;110:34–46.

82. Neufeld G, Mumbat Y, Smolkin T, Toledano S, Nir-Zvi I, Tzion K, Kessler O. The role of the semaphorins in cancer. Cell Adh Migr. 2010;1265–674.

Key Words: cardiac development • cardiovascular research • heart development