

# Chapter 9

## Semaphorin in the Heart

Masaki Ieda and Keiichi Fukuda

**Abstract** Semaphorin was cloned as a potent neural chemorepellent and is known as a directional guidance molecule for nerve fibers. However, recent studies demonstrated that semaphorin family members have important roles in cardiovascular development and diseases. The cardiovascular system consists of many types of cells, including neural cells, and is extensively innervated and tightly regulated by the autonomic nervous system. To maintain cardiac function properly, innervation density is strictly determined by the balance between neural chemoattractant and chemorepellent. Nerve growth factor, a potent chemoattractant, is abundantly synthesized from cardiomyocytes, but the chemorepellent for cardiac nerves was not determined until recently. We found that *Sema3a* is strongly expressed in the trabecular layer in early-stage embryos, but is reduced after birth, forming an epicardial-to-endocardial transmural sympathetic innervation patterning. Both *Sema3a*-deficient and -overexpressed mice showed sudden cardiac death and lethal arrhythmias from disruption of proper innervation patterning. More recently, Nakano et al. reported that a nonsynonymous polymorphism in *Sema3a* DNA is a risk factor for human cardiac arrest with ventricular fibrillation. Sympathetic innervation patterning was disrupted in these patients, suggesting proper expression of *Sema3a* and innervation patterning are critical to maintain arrhythmia-free hearts. *Sema3c* is expressed in the cardiac outflow tract during development and is important in cardiovascular patterning. *Gata6* regulates *Sema3c* expression, and disruption of the *Gata6/Sema3c* pathway leads to congenital heart disease in mouse and human. This review focuses on the recent progress of our understanding of heart development and disease, regulated by semaphorin family members, in mouse and human.

---

M. Ieda, M.D., Ph.D. (✉)

Department of Clinical and Molecular Cardiovascular Research, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

e-mail: [mieda@z8.keio.jp](mailto:mieda@z8.keio.jp)

K. Fukuda, M.D., Ph.D. (✉)

Department of Cardiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

e-mail: [kfukuda@a2.keio.jp](mailto:kfukuda@a2.keio.jp)

**Keywords** Heart • Cardiac nerve • Semaphorin • Arrhythmia • Congenital heart disease

## 9.1 Introduction

The heart consists of many types of cells, including cardiomyocytes, vascular cells, neural cells, and cardiac fibroblasts. Heart function is tightly regulated by the interactions between cardiomyocytes and other type of cells through secreting molecules. Understanding the cell–cell interactions and their molecular mechanisms during heart development might provide new therapeutic and preventive approaches for heart disease.

The relationship between autonomic nerve activity and arrhythmias is well recognized, but the molecular mechanisms determining the innervation densities and the consequence of its disruption were unclear. Compared with other organs, the heart is extensively innervated via the autonomic nervous system, which comprises the sympathetic and parasympathetic nerves. The sympathetic nervous system produces norepinephrine, and increases heart rate, conduction velocity, and myocardial contraction and relaxation. It is well known that sympathetic innervation density, which is abundant in the subepicardium and the central conduction system, is strictly determined within the heart (Ito and Zipes 1994; Crick et al. 1994, 1999a, b). The regional difference in sympathetic innervation influences specific cardiac functions to a different extent, effectively controlling heart rate and myocardial contraction and relaxation. Unbalanced sympathetic innervation might trigger lethal arrhythmia through ion channel modulation in cardiomyocytes (Cao et al. 2000; Dae et al. 1997; Qu and Robinson 2004). *Sema3a*, a class 3 secreted semaphorin, was cloned as a potent neural chemorepellent and a directional guidance molecule for nerve fibers. We found that *Sema3a* is expressed in cardiac tissues and determines sympathetic innervation patterning in the developing heart. Disruption of *Sema3a* expression in mouse hearts lead to fetal arrhythmias and sudden cardiac death. More recently, Nakano et al. reported that a nonsynonymous polymorphism in *Sema3a* is a risk factor for human unexplained cardiac arrest with ventricular fibrillation. They showed that sympathetic innervation patterning was disrupted in these patients, suggesting proper function of *Sema3a* is critical for arrhythmia-free hearts in humans.

Congenital heart diseases (CHD) occur in nearly 1 % of all live births and are the major cause of infant mortality and morbidity. Cardiac outflow tract (OFT) defects are estimated to account for approximately 30 % of CHD, but the genetic etiology of most OFT defects remains unknown. Increasing evidence has demonstrated that semaphorin 3C, a member of the semaphorin family of secreted factors, is expressed in the cardiovascular system during development. The semaphorin 3C knockout (KO) mouse revealed impaired migration of cardiac neural crest cells to the developing outflow tract, leading to OFT defects and perinatal death. *Gata6* directly

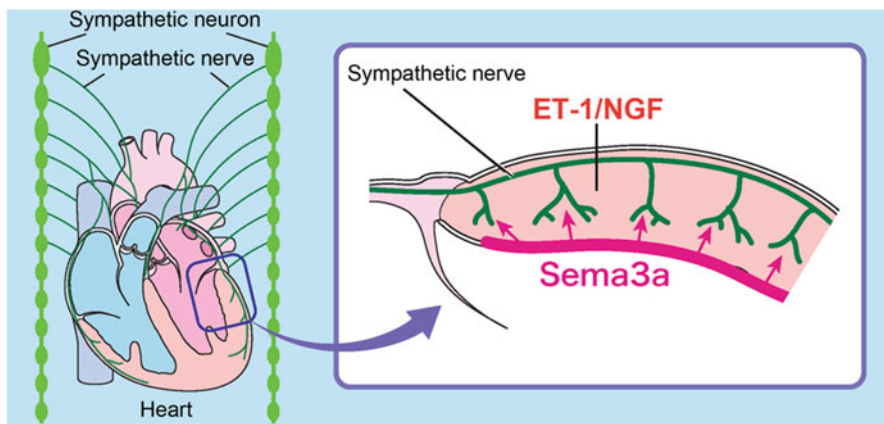
regulates semaphorin 3C expression during development, and neural crest-specific *Gata6* mutant mice revealed OFT defects, recapitulating the phenotype of the semaphorin 3C KO mouse. Kodo et al. found two mutations in *GATA6* in their series of 21 Japanese patients with persistent truncus arteriosus (PTA), a major disease in OFT defects. They demonstrated that both mutations disrupted the transcriptional activity of the *GATA6* protein, leading to failure of transactivation of *SEMA3C* and *PLXNA2*. The results in human OFT defects are consistent with those observed in *Gata6* and *Sema3c* mutant mice, demonstrating that the *GATA6/Sema3c* pathway is conserved among species and is critical for development of the cardiovascular system.

In this chapter, we review the function of semaphorin family members, demonstrated as important for cardiovascular development and disease in both mouse and human.

## 9.2 Cardiac Sympathetic Innervation and Nerve Growth Factor

The heart is innervated by sympathetic, parasympathetic, and sensory nerves derived from neural crest cells. Trunk neural crest cells migrate and form sympathetic ganglia by midgestation, subsequently proliferating and differentiating into mature neurons. The cardiac sympathetic nerves extend from the sympathetic neurons in stellate ganglia, which are located bilateral to the thoracic vertebrae. Sympathetic nerve fibers project from the base of the heart into the myocardium, and are located predominantly in the subepicardium of the ventricle (Fig. 9.1). The central conduction system, which includes the sinoatrial node, atrioventricular node, and His bundle, is abundantly innervated compared with the working myocardium. The regional difference in cardiac sympathetic innervation is highly conserved among mammals.

In general, the growth cone behavior of nerves is modulated by coincident signaling between neural chemoattractant and chemorepellent synthesized in the innervated tissue. Nerve growth factor (NGF), a potent neural chemoattractant, is a prototypic member of the neurotrophin family, members of which are critical for the differentiation, survival, and synaptic activity of the peripheral sympathetic and sensory nervous systems (Snider 1994; Lockhart et al. 1997; Brennan et al. 1999). We and others have reported that NGF expression in cardiomyocytes is critical as a sympathetic chemoattractant in normal and diseased hearts. Among several cardiac hypertrophic factors, we found that ET-1 is a key regulator of NGF expression in cardiomyocytes, and that the ET-1/NGF pathway is critical for sympathetic innervation in developing and right ventricular hypertrophy hearts (Ieda et al. 2004; Kimura et al. 2007). Given that ET-1 is strongly induced in myocardial infarction, the ET-1/NGF pathway might also be involved in NGF upregulation and nerve regeneration after myocardial infarction.



**Fig. 9.1** Inverse expression pattern of Sema3a and sympathetic innervation in developing hearts. The cardiac sympathetic nerves (green) extend from the sympathetic neurons in stellate ganglia, which are located bilateral to the thoracic vertebra (left). Cardiac sympathetic innervation shows an epicardial-to-endocardial transmural gradient (right). This patterning is established by the balance between ET-1/NGF and Sema3a expression in the heart. Note that NGF is abundantly expressed in the working myocardium, whereas Sema3a is specifically expressed in the subendocardium (Ieda et al. 2007, 2008)

### 9.3 Sema3a Is Critical for Cardiac Sympathetic Innervation Patterning

As already discussed, NGF, a neural chemoattractant, has critical functions in cardiac nerve development. In contrast, the neural chemorepellent that induces growth cone collapse and repels nerve axons was not identified in the heart. *Sema3a*, a class 3 secreted semaphorin, was cloned as a potent neural chemorepellent and a directional guidance molecule for nerve fibers (Puschel et al. 1995; Tanelian et al. 1997; Kawasaki et al. 2002). However, it was unknown whether cardiomyocytes produce Sema3a, and if so, whether this protein affects sympathetic neural patterning and cardiac performance.

We analyzed the time course and distribution of cardiac sympathetic innervation in developing mice ventricles (Ieda et al. 2007). TH-immunopositive sympathetic nerve endings appeared at embryonic day (E) 15 in the epicardial surface, and gradually increased in the myocardium after postnatal day (P) 7 and 42. In the ventricular myocardium, sympathetic nerves were richer in the subepicardium than the subendocardium, showing an epicardial-to-endocardial gradient (Crick et al. 1994; Ito and Zipes 1994; Chow et al. 1993; Hansson et al. 1998). To identify *Sema3a* expression and its relationship with innervation patterning in the heart, we analyzed the heterozygous *Sema3a* knocked-in *lacZ* mouse (*Sema3a<sup>lacZ/+</sup>*). At E12, *lacZ* expression was strongly detected in the heart, especially in the trabecular

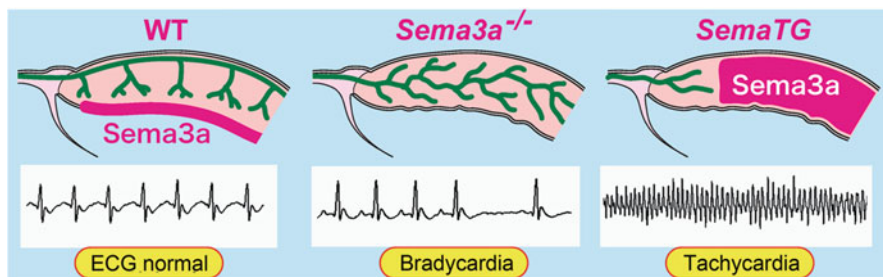
component of both ventricles. In E15 hearts, *lacZ* expression was observed at the subendocardium but not at the subepicardium in the atria and ventricles. At P1 and P42, *lacZ* expression was reduced in places and highlighted the Purkinje fiber network along the ventricular free wall (Tago et al. 1986; Kupersmidt et al. 1999). Quantitative RT-PCR of *Sema3a* in developing hearts also revealed *Sema3a* was detected from E12, and then decreased linearly in contrast to the sympathetic innervation. These results indicated that *Sema3a* has the opposite time-course and distribution of expression from sympathetic innervation in developing hearts, suggesting *Sema3a* might regulate cardiac innervation negatively (Fig. 9.1) (Ieda et al. 2008).

To investigate whether *Sema3a* is critical for cardiac sympathetic nerve development, we analyzed *Sema3a*-deficient mice (*Sema3a*<sup>-/-</sup>) (Behar et al. 1996; Taniguchi et al. 1997). The wild-type (WT) hearts showed a clear epicardial-to-endocardial gradient of sympathetic innervation; in contrast, sympathetic nerve density was reduced in the subepicardium but increased in the subendocardium in *Sema3a*<sup>-/-</sup> mice, resulting in disruption of the innervation gradient in *Sema3a*<sup>-/-</sup> ventricles. The *Sema3a*<sup>-/-</sup> mice also exhibited malformation of stellate ganglia that extended sympathetic nerves to the heart. To address whether the abnormal sympathetic innervation patterning in *Sema3a*<sup>-/-</sup> hearts was a secondary effect of the stellate ganglia malformation, we generated transgenic mice overexpressing *Sema3a* specifically in the heart (*SemaTG*) (Gulick et al. 1991). *SemaTG* mice were associated with reduced sympathetic innervation and attenuation of the epicardial-to-endocardial innervation gradient. These results indicated that cardiomyocyte-derived *Sema3a* has a critical role in cardiac sympathetic innervation by inhibiting neural growth. As cardiomyocyte-derived NGF acts as a chemoattractant, it might be the balance between NGF and *Sema3a* synthesized in the heart that determines cardiac sympathetic innervation patterning.

#### 9.4 *Sema3a* Maintains Arrhythmia-Free Hearts Through Sympathetic Innervation Patterning in Mouse

Most *Sema3a*<sup>-/-</sup> died within the first postnatal week; only 20 % remained viable until weaning (Taniguchi et al. 1997; Behar et al. 1996). To identify the cause of death and the effects of abnormal sympathetic neural distribution in *Sema3a*<sup>-/-</sup> hearts, we performed telemetric electrocardiography and heart rate variability analysis (Shusterman et al. 2002; Saba et al. 2003). In addition to multiple premature ventricular contractions, *Sema3a*<sup>-/-</sup> mice developed sinus bradycardia and abrupt sinus arrest as the result of sympathetic neural dysfunction.

The *SemaTG* mice died suddenly without any symptoms at 10 months of age. Sustained ventricular tachyarrhythmia was induced only in *SemaTG* mice but not in WT mice after epinephrine administration, and programmed electrical



**Fig. 9.2** Regulation of cardiac innervation patterning and arrhythmias. *Sema3a*-mediated proper sympathetic innervation patterning is critical to maintain arrhythmia-free hearts. *Sema3a*<sup>-/-</sup> mice exhibited sinus bradycardia, and *SemaTG* mice were highly susceptible to ventricular tachyarrhythmias (Ieda et al. 2007, 2008)

stimulation also revealed that *SemaTG* mice were highly susceptible to ventricular tachyarrhythmia (Wehrens et al. 2004; Kannankeril et al. 2006). The  $\beta$ -adrenergic receptor density was upregulated and the cAMP response after catecholamine injection was exaggerated in *SemaTG* ventricles. Action potential duration was significantly prolonged in hypoinnervated *SemaTG* ventricles, presumably via ion channel modulation. These results suggested that the higher susceptibility of *SemaTG* mice to ventricular arrhythmia was at least in part caused by catecholamine supersensitivity and action potential duration prolongation, either or both of which might augment triggered activity in cardiomyocytes (Kuo et al. 2001; Opthof et al. 1991; Priori and Corr 1990; Brunet et al. 2004; Costantini et al. 2005). Thus, *Sema3a*-mediated proper sympathetic innervation patterning is critical to maintain arrhythmia-free hearts (Fig. 9.2) (Ieda et al. 2008).

Sympathetic nerves modulate the function of ion channels and trigger various arrhythmias in diseased hearts (Qu and Robinson 2004; Dae et al. 1997). However, the relationship between sympathetic innervation and arrhythmogenicity in structurally normal hearts was unclear. *Sema3a*<sup>-/-</sup> mice exhibited sinus bradycardia, abrupt sinus slowing, and stellate ganglia defects. Consistent with our data, right stellectomy induced sinus bradycardia and sudden asystolic death in dogs (Sosunov et al. 2001). Stramba-Badiale et al. also reported that a developmental abnormality in cardiac innervation might be involved role in the genesis of some cases of sudden infant death syndrome (Stramba-Badiale et al. 1992). The *SemaTG* hearts were also highly susceptible to ventricular arrhythmias, although without contractile dysfunction or structural defects. Given that catecholamine augments systolic function, it is surprising that *SemaTG* mice showed normal cardiac function. However, patients who underwent heart transplantation and had denervated hearts did not show heart failure, whereas about 10 % of the patients developed sudden cardiac death, presumably because of arrhythmias (Chantranuwat et al. 2004). These results suggested that regulation of cardiac nerves should be a new paradigm in the management of sudden cardiac death.

## 9.5 The Crosstalk Between Neurotrophin and Semaphorin Signaling in Nerve Development

Because cardiomyocyte-derived NGF acts as a chemoattractant, it is possible that the balance between NGF and Sema3a synthesized in the heart determines cardiac sympathetic innervation patterning. The growth cone behavior of somatic sensory axons is modulated by coincident signaling between NGF and Sema3a (Tang et al. 2004; Kitsukawa et al. 1997), both of which are expressed within the developing spinal cord and influence pathway guidance of sensory axons during development. Sema3a is specifically expressed in the ventral half of the spinal cord and mediates NGF-responsive sensory axons to terminate at the dorsal part of the spinal cord (Puschel et al. 1995; Wright et al. 1995). In addition, targeted inactivation of *Sema3a* disrupts neural patterning and projections in the spinal cord, further highlighting the importance of Sema3a signaling for the directional guidance of nerve fibers (Taniguchi et al. 1997; Behar et al. 1996).

However, the interaction of NGF and Sema3A signaling is further complicated as the low-affinity NGF receptor p75 can partner with the Sema3A receptors neuropilin-1 and plexin A4 to attenuate their combined ability to repel growing axons (Ben-Zvi et al. 2007). Lorentz et al. found that the subendocardium innervation of adult p75 knockout ventricles was disrupted, with the left ventricle essentially devoid of sympathetic nerve fibers, whereas innervation density of the subepicardium was normal (Lorentz et al. 2010). This neural patterning defect is similar to that seen in mice overexpressing Sema3A, such that the sympathetic axons lacking p75 are highly sensitive to Sema3A-mediated inhibition of neurite outgrowth. The heterogeneous innervation was also associated with altered cardiac  $\beta$ 1-adrenergic receptor expression and sensitivity, and a significant increase in spontaneous ventricular arrhythmias, which were also observed in Sema3A-overexpressing mice. These results suggested that as sympathetic neurons are innervating the heart, p75, a receptor for neurotrophins, acts to blunt the repulsive effects of Sema3A from the subendocardium, thereby allowing axonal arborization and orchestrating a highly organized epicardial-to-endocardial innervation gradient in the ventricle (Carter et al. 2010).

## 9.6 A Nonsynonymous Polymorphism in Sema3a Is a Risk Factor for Human Unexplained Cardiac Arrest with Ventricular Fibrillation

Unexplained cardiac arrest (UCA) with documented ventricular fibrillation is defined as spontaneous ventricular fibrillation (VF) that is not associated with known structural or electrical heart diseases and is one of the major causes of sudden cardiac death. The relationship between abnormal autonomic nerve activity and lethal ventricular arrhythmias is well known, but the molecular mechanism deter-

mining the innervation densities in patients with lethal arrhythmia was not clarified because of the high mortality rate and difficulty of diagnosis. Nakano et al. reported that UCA patients with spontaneous VF had a high incidence of a polymorphism in the semaphorin 3A gene (Nakano et al. 2013). A nonsynonymous polymorphism (I334V, rs138694505A>G) in exon 10 of the SEMA3A gene identified through resequencing was significantly associated with UCA (combined  $P = 0.0004$ , OR 3.08, 95% CI 1.67–5.7). Overall, 15.7 % of UCA patients carried the risk genotype G, whereas only 5.6 % of controls did so. In patients with SEMA3AI334V, VF predominantly occurred at rest during the night. They showed sinus bradycardia and sinus dysfunction, and their RR intervals on electrocardiography tended to be longer than those in patients without SEMA3AI334V ( $P = 0.039$ ), which is consistent with the *Sema3A* KO mouse phenotype (Ieda et al. 2007). Immunofluorescence staining of cardiac biopsy specimens revealed that sympathetic nerves were absent in the subendocardial layer in normal hearts and in patients without SEMA3AI334V. In patients with SEMA3AI334V, in contrast, sympathetic nerves were distributed in the subendocardial layer. This finding was consistently observed in patients with SEMA3AI334V ( $n = 4$ ) but not in those without SEMA3AI334V ( $n = 8$ ), suggesting disruption of the epicardial-to-endocardial transmural sympathetic innervation patterning in the heart of UCA patients with SEMA3AI334V. On the other hand, NGF, a neural attractant factor, was similarly expressed in the subendocardial layer in patients with and without SEMA3AI334V. The dorsal root ganglion (DRG) repulsion assay revealed that the axon-repelling and axon-collapsing activities of mutant SEMA3AI334V genes were significantly weaker than those of wild-type SEMA3A genes. These results indicate that SEMA3AI334V disrupts the SEMA3A function of inhibiting neural growth and impaired appropriate innervation patterning, and that SEMA3AI334V is a risk factor for human UCA and contributes to the pathogenesis of UCA.

## 9.7 The *Gata6*/*Sema3c* Pathway Is Critical for Cardiovascular Morphogenesis in the Mouse

The cardiac neural crest plays a critical role in patterning the vertebrate vascular system (Creazzo et al. 1998). During early embryogenesis (E8.0–9.0 in the mouse), the cardiac neural crest arises from the dorsal neural tube and migrates ventrally to populate the aortic arch arteries and cardiac outflow tract. These neural crest-derived cells respond to poorly characterized developmental cues to differentiate into vascular smooth muscle cells (VSMCs) and contribute to vascular formation during development. Semaphorin 3C is a member of the semaphorin family of secreted factors that are known to be important in regulating neuronal as well as vascular patterning. During murine embryonic development, semaphorin 3C is expressed in the mesenchyme surrounding the developing aortic arch arteries and in neural crest-derived SMCs. These cells also express neuropilin 1 and plexin-



A2, the components of a potential heterodimeric semaphorin 3C receptor. Targeted inactivation of semaphorin 3C causes impaired migration of neural crest cells to the developing cardiac outflow tract, resulting in interruption of the aortic arch and persistent truncus arteriosus (Feiner et al. 2001; Brown et al. 2001). It has been proposed that semaphorin 3C acts as a guidance molecule, regulating migration of neural crest cells that express semaphorin receptors such as plexin-A2, but the molecular mechanisms regulating *Sema3c* expression and disruption of this pathway in human hearts were not clear until recently. Toyofuku et al. reported that the coordination of repulsive cues provided by *Sema6A/Sema6B* through plexin-A2 paired with the attractive cue by *Sema3C* through plexin-D1 is required for the precise navigation of migrating cardiac neural crest cells to the cardiac outflow tract (Toyofuku et al. 2008).

Lepore et al. demonstrated that GATA-6 regulates semaphorin 3C expression in the neural crest cells, and this pathway regulates morphogenetic patterning of the aortic arch and cardiac outflow tract during development in the mouse (Lepore et al. 2006). Conditional inactivation of GATA-6 in vascular smooth muscle cells resulted in perinatal mortality from a spectrum of cardiovascular defects, including interrupted aortic arch and persistent truncus arteriosus. Inactivation of GATA-6 in neural crest cells recapitulated these abnormalities, demonstrating a cell-autonomous requirement for GATA-6 in neural crest-derived SMCs. Importantly, deletion of GATA-6 in neural crest cells resulted in an absence of detectable semaphorin 3C- or plexin-A2-expressing cardiac neural crest cells within the conotruncal endocardial cushions that contribute to aorticopulmonary septation. These results are consistent with the phenotypes observed in semaphorin 3C-deficient mice (Feiner et al. 2001; Brown et al. 2001), the impairment of neural crest cell migration into the cardiac outflow tract.

## 9.8 Gata6 Mutations Cause Human Congenital Heart Disease by Disrupting *Sema3c*/Plexin-A2 Signaling

Congenital heart diseases (CHD) occur in nearly 1 % of all live births and are the major cause of infant mortality and morbidity (Hoffman and Kaplan 2002). Although an improved understanding of the genetic causes of CHD would provide insight into the underlying pathobiology, the genetic etiology of most CHD remains unknown. Cardiac outflow tract (OFT) defects are estimated to account for approximately 30 % of CHD and usually require an intervention during the first year of life. Kodo et al. reported the identification and characterization of two mutations of GATA6 DNA (9.5 %) in their series of 21 Japanese patients with persistent truncus arteriosus (PTA), a form of OFT defect (Kodo et al. 2009). Both mutations disrupted the transcriptional activity of the GATA6 protein on downstream target genes involved in the development of the outflow tract. They also confirmed that the expression of *SEMA3C* and *PLXNA2* in the developing OFT was regulated directly

through the consensus GATA binding sites conserved between human and mouse, in vitro and in vivo. Mutant GATA6 proteins failed to transactivate SEMA3C and PLXNA2, and mutation of the GATA sites on enhancer elements of *Sema3c* and *Plxn2* abolished their activity, suggesting that mutations of GATA6 cause specific forms of human CHDs. These results are consistent with the mouse phenotype observed in semaphorin 3C-deficient mice and conditional inactivation of GATA-6 in neural crest cells and VSMCs, as previously discussed.

## 9.9 Conclusions

The heart consists of many types of cells, including cardiomyocytes, vascular cells, and neural cells, which tightly regulate heart development and cardiac function. Semaphorin family members are critical in the interactions between cardiomyocytes and other types of cells (Fig. 9.3). Cardiac nerves are highly plastic, and innervation patterning is strictly controlled by the balance between NGF and *Sema3a* synthesized in the heart. *Sema3a* inhibits neural growth and establishes proper innervation patterning in the heart. The disruption of sympathetic innervation patterning by *Sema3a* misexpression may lead to sudden cardiac death and fetal arrhythmias in both mouse and human (Ieda et al. 2007; Nakano et al. 2013). Cardiac outflow tract formation is organized by the interaction of migrating cardiac neural crest cells and surrounding tissues. The GATA6/*Sema3c* signaling pathway is conserved among mammals, and its disruption results in congenital heart diseases in humans.

		Sema3a	Ref.	Gata6/Sema3c	Ref.
Mouse	Function	Sympathetic innervation patterning	Ieda et al. Lorentz et al.	Cardiac outflow tract development	Feiner et al. Brown et al.
	Mutant mice	Disruption of sympathetic innervation Fetal arrhythmias and sudden death	Ieda et al.	Cardiac outflow tract defects (interrupted aortic arch and persistent truncus arteriosus)	Lepore et al.
Human		A polymorphism in Sema3A Cardiac arrest and ventricular fibrillation	Nakano et al.	Two mutations in Gata6 Persistent truncus arteriosus	Kodo et al.

**Fig. 9.3** Summary of cardiac defects mediated by *Sema3a* and *Sema3c* signaling. *Sema3a* is important for cardiac sympathetic innervation and maintains arrhythmia-free hearts, and *Gata6/sem3c* is critical for proper cardiac outflow tract formation (*Ref.* indicates references in the text)

Understanding of the divergent effects of semaphorin family members on cardiac innervation and cardiovascular patterning might thus represent a novel step toward potential therapies for heart diseases.

**Acknowledgments** This study was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation.

## References

- Behar O, Golden JA, Mashimo H, Schoen FJ, Fishman MC (1996) Semaphorin III is needed for normal patterning and growth of nerves, bones and heart. *Nature (Lond)* 383(6600):525–528
- Ben-Zvi A, Ben-Gigi L, Klein H, Behar O (2007) Modulation of semaphorin3A activity by p75 neurotrophin receptor influences peripheral axon patterning. *J Neurosci* 27(47):13000–13011. doi:27/47/13000 [pii]
- Brennan C, Rivas-Plata K, Landis SC (1999) The p75 neurotrophin receptor influences NT-3 responsiveness of sympathetic neurons in vivo. *Nat Neurosci* 2(8):699–705
- Brown CB, Feiner L, Lu MM, Li J, MA X, Webber AL, Jia L, Raper JA, Epstein JA (2001) PlexinA2 and semaphorin signaling during cardiac neural crest development. *Development (Camb)* 128(16):3071–3080
- Brunet S, Aimond F, Li H, Guo W, Eldstrom J, Fedida D, Yamada KA, Nerbonne JM (2004) Heterogeneous expression of repolarizing, voltage-gated K<sup>+</sup> currents in adult mouse ventricles. *J Physiol* 559(pt 1):103–120
- Cao JM, Fishbein MC, Han JB, Lai WW, Lai AC, Wu TJ, Czer L, Wolf PL, Denton TA, Shintaku IP, Chen PS, Chen LS (2000) Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. *Circulation* 101(16):1960–1969
- Carter BD, Feng N, Paolucci N (2010) The p75 neurotrophin receptor, semaphorins, and sympathetic traffic in the heart. *Am J Physiol Heart Circ Physiol* 298(6):H1633–H1636
- Chantranuwat C, Blakey JD, Kobashigawa JA, Moriguchi JD, Laks H, Vassilakis ME, Fishbein MC (2004) Sudden, unexpected death in cardiac transplant recipients: an autopsy study. *J Heart Lung Transplant* 23(6):683–689
- Chow LT, Chow SS, Anderson RH, Gosling JA (1993) Innervation of the human cardiac conduction system at birth. *Br Heart J* 69(5):430–435
- Costantini DL, Arruda EP, Agarwal P, Kim KH, Zhu Y, Zhu W, Lebel M, Cheng CW, Park CY, Pierce SA, Guerchicoff A, Pollevick GD, Chan TY, Kabir MG, Cheng SH, Husain M, Antzelevitch C, Srivastava D, Gross GJ, Hui CC, Backx PH, Bruneau BG (2005) The homeodomain transcription factor *Irx5* establishes the mouse cardiac ventricular repolarization gradient. *Cell* 123(2):347–358
- Creazzo TL, Godt RE, Leatherbury L, Conway SJ, Kirby ML (1998) Role of cardiac neural crest cells in cardiovascular development. *Annu Rev Physiol* 60:267–286. doi:10.1146/annurev.physiol.60.1.267
- Crick SJ, Wharton J, Sheppard MN, Royston D, Yacoub MH, Anderson RH, Polak JM (1994) Innervation of the human cardiac conduction system. A quantitative immunohistochemical and histochemical study. *Circulation* 89(4):1697–1708
- Crick SJ, Anderson RH, Ho SY, Sheppard MN (1999a) Localisation and quantitation of autonomic innervation in the porcine heart. II: Endocardium, myocardium and epicardium. *J Anat* 195(Pt 3):359–373
- Crick SJ, Sheppard MN, Ho SY, Anderson RH (1999b) Localisation and quantitation of autonomic innervation in the porcine heart. I: Conduction system. *J Anat* 195(Pt 3):341–357

- Dae MW, Lee RJ, Ursell PC, Chin MC, Stillson CA, Moise NS (1997) Heterogeneous sympathetic innervation in German shepherd dogs with inherited ventricular arrhythmia and sudden cardiac death. *Circulation* 96(4):1337–1342
- Feiner L, Webber AL, Brown CB, Lu MM, Jia L, Feinstein P, Mombaerts P, Epstein JA, Raper JA (2001) Targeted disruption of semaphorin 3C leads to persistent truncus arteriosus and aortic arch interruption. *Development (Camb)* 128(16):3061–3070
- Gulick J, Subramaniam A, Neumann J, Robbins J (1991) Isolation and characterization of the mouse cardiac myosin heavy chain genes. *J Biol Chem* 266(14):9180–9185
- Hansson M, Kjorell U, Forsgren S (1998) Increased immunoreexpression of atrial natriuretic peptide in the heart conduction system of the rat after cardiac sympathectomy. *J Mol Cell Cardiol* 30(10):2047–2057
- Hoffman JJ, Kaplan S (2002) The incidence of congenital heart disease. *J Am Coll Cardiol* 39(12):1890–1900
- Ieda M, Fukuda K, Hisaka Y, Kimura K, Kawaguchi H, Fujita J, Shimoda K, Takeshita E, Okano H, Kurihara Y, Kurihara H, Ishida J, Fukamizu A, Federoff HJ, Ogawa S (2004) Endothelin-1 regulates cardiac sympathetic innervation in the rodent heart by controlling nerve growth factor expression. *J Clin Invest* 113(6):876–884
- Ieda M, Kanazawa H, Kimura K, Hattori F, Ieda Y, Taniguchi M, Lee JK, Matsumura K, Tomita Y, Miyoshi S, Shimoda K, Makino S, Sano M, Kodama I, Ogawa S, Fukuda K (2007) *Sema3a* maintains normal heart rhythm through sympathetic innervation patterning. *Nat Med* 13(5):604–612
- Ieda M, Kimura K, Kanazawa H, Fukuda K (2008) Regulation of cardiac nerves: a new paradigm in the management of sudden cardiac death? *Curr Med Chem* 15(17):1731–1736
- Ito M, Zipes DP (1994) Efferent sympathetic and vagal innervation of the canine right ventricle. *Circulation* 90(3):1459–1468
- Kannankeril PJ, Mitchell BM, Goonasekera SA, Chelu MG, Zhang W, Sood S, Kearney DL, Danila CI, De Biasi M, Wehrens XH, Pautler RG, Roden DM, Taffet GE, Dirksen RT, Anderson ME, Hamilton SL (2006) Mice with the R176Q cardiac ryanodine receptor mutation exhibit catecholamine-induced ventricular tachycardia and cardiomyopathy. *Proc Natl Acad Sci U S A* 103(32):12179–12184
- Kawasaki T, Bekku Y, Suto F, Kitsukawa T, Taniguchi M, Nagatsu I, Nagatsu T, Itoh K, Yagi T, Fujisawa H (2002) Requirement of neuropilin 1-mediated *Sema3A* signals in patterning of the sympathetic nervous system. *Development (Camb)* 129(3):671–680
- Kimura K, Ieda M, Kanazawa H, Yagi T, Tsunoda M, Ninomiya S, Kurosawa H, Yoshimi K, Mochizuki H, Yamazaki K, Ogawa S, Fukuda K (2007) Cardiac sympathetic rejuvenation: a link between nerve function and cardiac hypertrophy. *Circ Res* 100(12):1755–1764
- Kitsukawa T, Shimizu M, Sanbo M, Hirata T, Taniguchi M, Bekku Y, Yagi T, Fujisawa H (1997) Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice. *Neuron* 19(5):995–1005
- Kodo K, Nishizawa T, Furutani M, Arai S, Yamamura E, Joo K, Takahashi T, Matsuoka R, Yamagishi H (2009) *GATA6* mutations cause human cardiac outflow tract defects by disrupting semaphorin-plexin signaling. *Proc Natl Acad Sci U S A* 106(33):13933–13938. doi:10.1073/pnas.0904744106
- Kuo HC, Cheng CF, Clark RB, Lin JJ, Lin JL, Hoshijima M, Nguyen-Tran VT, Gu Y, Ikeda Y, Chu PH, Ross J, Giles WR, Chien KR (2001) A defect in the Kv channel-interacting protein 2 (*KChIP2*) gene leads to a complete loss of *I(to)* and confers susceptibility to ventricular tachycardia. *Cell* 107(6):801–813
- Kupersmidt S, Yang T, Anderson ME, Wessels A, Niswender KD, Magnuson MA, Roden DM (1999) Replacement by homologous recombination of the *minK* gene with *lacZ* reveals restriction of *minK* expression to the mouse cardiac conduction system. *Circ Res* 84(2):146–152
- Lepore JJ, Mericko PA, Cheng L, Lu MM, Morrisey EE, Parmacek MS (2006) *GATA-6* regulates semaphorin 3C and is required in cardiac neural crest for cardiovascular morphogenesis. *J Clin Invest* 116(4):929–939. doi:10.1172/JCI27363
- Lockhart ST, Turrigiano GG, Birren SJ (1997) Nerve growth factor modulates synaptic transmission between sympathetic neurons and cardiac myocytes. *J Neurosci* 17(24):9573–9582

- Lorentz CU, Alston EN, Belcik T, Lindner JR, Giraud GD, Habecker BA (2010) Heterogeneous ventricular sympathetic innervation, altered beta-adrenergic receptor expression, and rhythm instability in mice lacking the p75 neurotrophin receptor. *Am J Physiol Heart Circ Physiol* 298(6):H1652–H1660. doi:01128.2009 [pii]
- Nakano Y, Chayama K, Ochi H, Toshishige M, Hayashida Y, Miki D, Hayes CN, Suzuki H, Tokuyama T, Oda N, Suenari K, Uchimura-Makita Y, Kajihara K, Sairaku A, Motoda C, Fujiwara M, Watanabe Y, Yoshida Y, Ohkubo K, Watanabe I, Nogami A, Hasegawa K, Watanabe H, Endo N, Aiba T, Shimizu W, Ohno S, Horie M, Arihiro K, Tashiro S, Makita N, Kihara Y (2013) A nonsynonymous polymorphism in semaphorin 3A as a risk factor for human unexplained cardiac arrest with documented ventricular fibrillation. *PLoS Genet* 9(4):e1003364. doi:10.1371/journal.pgen.1003364
- Ophthof T, Misier AR, Coronel R, Vermeulen JT, Verberne HJ, Frank RG, Moulijn AC, van Capelle FJ, Janse MJ (1991) Dispersion of refractoriness in canine ventricular myocardium. Effects of sympathetic stimulation. *Circ Res* 68(5):1204–1215
- Priori SG, Corr PB (1990) Mechanisms underlying early and delayed afterdepolarizations induced by catecholamines. *Am J Physiol* 258(6 Pt 2):H1796–H1805
- Puschel AW, Adams RH, Betz H (1995) Murine semaphorin D/collapsin is a member of a diverse gene family and creates domains inhibitory for axonal extension. *Neuron* 14(5):941–948
- Qu J, Robinson RB (2004) Cardiac ion channel expression and regulation: the role of innervation. *J Mol Cell Cardiol* 37(2):439–448
- Saba S, London B, Ganz L (2003) Autonomic blockade unmasks maturational differences in rate-dependent atrioventricular nodal conduction and facilitation in the mouse. *J Cardiovasc Electrophysiol* 14(2):191–195
- Shusterman V, Usiene I, Harrigal C, Lee JS, Kubota T, Feldman AM, London B (2002) Strain-specific patterns of autonomic nervous system activity and heart failure susceptibility in mice. *Am J Physiol Heart Circ Physiol* 282(6):H2076–H2083
- Snider WD (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* 77(5):627–638
- Sosunov EA, Anyukhovskiy EP, Gainullin RZ, Plotnikov A, Danilo P Jr, Rosen MR (2001) Long-term electrophysiological effects of regional cardiac sympathetic denervation of the neonatal dog. *Cardiovasc Res* 51(4):659–669
- Stramba-Badiale M, Lazzarotti M, Schwartz PJ (1992) Development of cardiac innervation, ventricular fibrillation, and sudden infant death syndrome. *Am J Physiol* 263(5 pt 2):H1514–H1522
- Tago H, Kimura H, Maeda T (1986) Visualization of detailed acetylcholinesterase fiber and neuron staining in rat brain by a sensitive histochemical procedure. *J Histochem Cytochem* 34(11):1431–1438
- Tanelian DL, Barry MA, Johnston SA, Le T, Smith GM (1997) Semaphorin III can repulse and inhibit adult sensory afferents in vivo. *Nat Med* 3(12):1398–1401
- Tang XQ, Tanelian DL, Smith GM (2004) Semaphorin3A inhibits nerve growth factor-induced sprouting of nociceptive afferents in adult rat spinal cord. *J Neurosci* 24(4):819–827
- Taniguchi M, Yuasa S, Fujisawa H, Naruse I, Saga S, Mishina M, Yagi T (1997) Disruption of semaphorin III/D gene causes severe abnormality in peripheral nerve projection. *Neuron* 19(3):519–530
- Toyofuku T, Yoshida J, Sugimoto T, Yamamoto M, Makino N, Takamatsu H, Takegahara N, Suto F, Hori M, Fujisawa H, Kumanogoh A, Kikutani H (2008) Repulsive and attractive semaphorins cooperate to direct the navigation of cardiac neural crest cells. *Dev Biol* 321(1):251–262. doi:10.1016/j.ydbio.2008.06.028
- Wehrens XH, Lehnart SE, Reiken SR, Deng SX, Vest JA, Cervantes D, Coromilas J, Landry DW, Marks AR (2004) Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. *Science* 304(5668):292–296
- Wright DE, White FA, Gerfen RW, Silos-Santiago I, Snider WD (1995) The guidance molecule semaphorin III is expressed in regions of spinal cord and periphery avoided by growing sensory axons. *J Comp Neurol* 361(2):321–333