Overexpression of sema3a in myocardial infarction border zone decreases vulnerability of ventricular tachycardia post-myocardial infarction in rats

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Abstract

The expression of the chemorepellent Sema3a is inversely related to sympathetic innervation. We investigated whether overexpression of Sema3a in the myocardial infarction (MI) border zone could attenuate sympathetic hyper-innervation and decrease the vulnerability to malignant ventricular tachyarrhythmia (VT) in rats. Survived MI rats were randomized to phosphate buffered saline (PBS, n = 12); mock lentivirus (MLV, n = 13) and lentivirus-mediated overexpression of Sema3a (SLV, n = 13) groups. Sham-operated rats served as control group (CON, n = 20). Cardiac function and electrophysiological study (PES) were performed at 1 week later. Blood and tissue samples were collected for histological analysis, epinephrine (EPI), growth-associated factor 43 (GAP43) and tyrosine hydroxylase (TH) measurements. QTc intervals were significantly shorter in SLV group than in PBS and MLV groups (168.6 ± 7.8 vs. 178.1 ± 9.5 and 180.9 ± 8.2 ms, all P < 0.01). Inducibility of VT by PES was significantly lower in the SLV group [30.8% (4/13)] than in PBS [66.7% (8/12)] and MLV [61.5% (8/13)] groups (P < 0.05). mRNA and protein expressions of Sema3a were significantly higher and the protein expression of GAP43 and TH was significantly lower at 7 days after transduction in SLV group compared with PBS, MLV and CON groups. Myocardial EPI in the border zone was also significantly lower in SLV group than in PBS and MLV group (8.73 ± 1.30 vs. 11.94 ± 1.71 and 12.24 ± 1.54 μg/g protein, P < 0.001). Overexpression of Sema3a in MI border zone could reduce the inducibility of ventricular arrhythmias by reducing sympathetic hyper-reinnervation after infarction.

Keywords: myocardial infarction • Sema3a • reinnervation • ventricular tachyarrhythmia

Introduction

Sudden cardiac death (SCD) remains a major and unresolved public health problem. Previous myocardial infarction (MI) was identified in 75% of SCD victims [1, 2]. In most cases, the direct cause of SCD is ventricular fibrillation (VF), which is usually preceded by ventricular tachycardia (VT). Life-threatening ventricular arrhythmia is thus an important cause of mortality post-MI [3, 4]. Previous studies demonstrated that sympathetic hyper-innervation was associated with arrhythmogenesis and SCD and augmented sympathetic nerve regeneration could increase the incidence of VT, VF and SCD in chronic MI animal models [5–10].

Semaphorins are a family of secreted and membrane-anchored glycoproteins [11] and are important in repulsive axon guidance during neuroembryogenesis [12]. Sema3a, one of the best characterized members in this family, is a diffusible molecule which could induce growth cone collapse and axon repulsion of several neuronal populations [13–16]. Down-regulated expression of Sema3a mRNA in adult neurons was observed in post-peripheral nerve injury [17, 18] and down-regulation of semaphorin expression could result in a more permissive environment for axonal regeneration and sprouting.

In transgenic mice, Sema3a expression was inversely related to sympathetic innervation, cardiac-specific Sema3a overexpression reduced sympathetic innervation, however, increased susceptibility to
ventricular arrhythmias [19]. It remains unknown now whether local overexpression Sema3a in the MI border zone may decrease or increase the susceptibility to ventricular arrhythmias. We observed the effect of Sema3a overexpression in the MI border zone on the susceptibility to ventricular arrhythmias in a rat MI model.

Materials and methods

Lentivirus production

A third generation of self-inactivating lentivirus vector was purchased from Shanghai Innovation Biotechnology Co. Ltd (Shanghai, China), which contains a cytomegalovirus -driven green-fluorescent protein (GFP) reporter gene and an EF1-α-β promoter upstream of cloning restriction sites (ClaI and mluI) to allow introduction of oligonucleotides encoding short hairpin RNAs (shRNAs). The sequence of the shRNA targeting rat Sema3a, 5′-GATGAGTTCCTACTGCTCAC-3′, has previously been proven to efficiently down-regulate rat Sema3a [20]. A nonsense shRNA, with the sense sequence 5′-CGACGAGAAGGATTGAAA-3′ which lacks complementary sequences in the murine genome, was used as a control. A BLAST search was carried out to avoid unintentional silencing of non-target host cell genes (www.ncbi.nlm.nih.gov). Oligos were chemically synthesized, annealed and cloned into the shRNA lentivector between the ClaI and mluI sites of the plasmid. Correct insertions of shRNA cassettes were confirmed by direct DNA sequencing. GeneBank accession number is 017310. Recombinant lentivirus was produced by cotransfecting 293T cells with the lentivirus expression plasmid and packaging plasmids using calcium phosphate method [21]. Infectious titre was harvested at 72 hrs after transfection, centrifuged to eliminate cell debris and then filtered through 0.22 μm cellulose acetate filters. Infectious titre was determined by fluorescence-activated cell sorting analysis of GFP positive in 293T cells. Virus titres were in the range of 10^8 transducing units/ml medium.

MI and Lentiviral vector microinjection

Sixty male adult Sprague–Dawley rats were randomized to three groups and subjected to MI operation (n = 20 each): (i) phosphate buffered saline (PBS); (ii) mock lentivirus (MLV) and (iii) lentivirus-mediated overexpression of Sema3a (SLV). MI was induced by ligation of the left anterior descending (LAD) coronary artery as previously described [22]. Ligation was deemed successful when the anterior wall of the left ventricle turned pale. Six hours post-MI, six intramyocardial PBS, MLV and SLV injections at MI border zone (50 μl each) were performed under direct observation using a 31-gauge needle in rats with echocardiographic-determined left ventricular ejection fraction (EF) <50%. Reporter gene expression was verified by fluorescence microscopy after 1 week for identifying transduced lentiviral vector in the recipient heart. The observation duration was set at 1 week post-MI because sympathetic reinnervation has been shown to be present at 6 days after injury [23]. Sham-operated rats served as normal control group (CON, n = 20). The animal experiment was approved by the medical ethics committee of Xinhua Hospital, Shanghai Jiaotong University, School of Medicine, and conforms to the Principles of Laboratory Animal Care (National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (NIH). All evaluations were carried out in a blinded manner.

Assessment of cardiac function by echocardiography

Left ventricular (LV) ejection fraction (EF) was measured with M-mode (Veo 770 System, and 15-MHz probe, VisualSonics Inc, Toronto, ON, Canada) at baseline, at 5 hrs and at 1 week post-LAD ligation by single operator blinded to the experimental designs in sedated rats with pentobarbital (40 mg/kg i.p; Pentobarbital sodium, Fiuka, Sweden).

Ventricular PES in sedated rats

After echocardiographic examination, all rats underwent programmed electrical stimulation (PES) study by an operator blinded to the experimental designs. Surface six-lead ECGs were recorded using 25-gauge subcutaneous electrodes connected to a recording system through an analogue-digital converter for monitoring and off-line analyses. ECG channels were filtered out below 10 Hz and above 100 Hz. Standard criteria were used for interval measurements: RR, PR, QRS and QT, and QTc (QT interval corrected for the heart rate using Bazett’s formula). A 1.9 F eight-pole catheter for small animal electrophysiology (Science, Ontario, Canada) was advanced from the right external jugular vein to the right atrium and through the tricuspid valve to the right ventricle. Pacing was performed by applying a 1-ms pulse pacing of width at two times higher than capture threshold. Standard clinical PES protocols were used, including burst, single, double and triple extra stimuli applied following a train of nine stimuli at 100-ms drive cycle length. The coupling interval of the last extra stimulus was decreased by 2-ms steps from 80 ms down to the ventricular effective refractory period (VERP). PES protocols were interrupted if sustained VT or VF was induced. Sustained VT was defined as fast ventricular rhythm of 15 or more beats, according to Lambeth Conventions [24]. No discrimination was made between monomorphic and polymorphic VT. Arrhythmia scoring system established by Curtis et al. was used (Table 1) [25].

Tissue preparation

Post-PES all rats were killed in deep anaesthesia with overdose pentobarbital (80 mg/kg bodyweight) and hearts were excised and rinsed in cold PBS. For histological analysis, hearts were perfused fixed with 4% paraformaldehyde, and frozen at 4°C. For cryosection, fresh tissues were snap frozen in liquid nitrogen, embedded in OCT compound in cryomoulds and stored at −80°C. For Western blot analysis and ELISA,
ELISA for epinephrine (EPI) levels

Blood samples of rats were collected before PES and immediately centrifuged at 3,000 × g for 10 min, and the serum was stored at −80°C until analysis. EPI levels of serum and MI border zone tissue protein extraction were measured using a commercial ELISA kit (R&D, Minneapolis, MN, USA).

Table 2 Primary and secondary antibodies for Western blot analysis used

| Antibody Type | Company                          | Dilution |
|---------------|----------------------------------|----------|
| Primary Antibody | Rabbit anti-human Sema3A antibody | Santa Cruz | 1:200  |
|                | Rabbit anti-rat Tyrosine Hydroxylase antibody | Enzo Life Sciences | 1:500  |
|                | Rabbit anti-rat Growth-associated Factor 43 antibody | Abcom | 1:5000  |
| Secondary Antibody | Goat anti-rabbit HRP conjugated | Jackson Immuno-Research | 1:1000  |

Histological analysis

Five micrometre sections were cut, stained with Masson’s trichrome and mounted. The inner and outer diameters of the LV were traced with a digital image processing system (Leica Qwin V3, Wetzels, Germany). Infarct size (%) was expressed as the mean of the percentage of infarcted LV versus total LV inner and outer circumferences [26].

Immunostaining for visualization of sympathetic innervation

An indirect immunofluorescence technique was adopted for the visualization of sympathetic innervation. Cryostat sections (5 μm thick) were cut and mounted on silica gel-coated slides, stored at −80°C until use. Before staining, slides were warmed at room temperature for 1 hr and fixed in ice-cold acetone for 10 min., air dried for 1 hr and washed in PBS and proceeded to standard staining procedure. Cryostat sections stained with antibodies to β-actinin (Santa Cruz, Dallas, TX, USA), GAP43 (a marker peptide for neuronal regeneration and outgrowth, Abcom Ltd, Hongkong) and TH (Enzo Life Sciences, Farmingdale, NY, USA) to detect cardiomyocytes and sympathetic nerve fibres respectively. The sections were incubated with secondary antibodies conjugated with Rhodamine (Jackson Immuno Research, West Grove, PA, USA) and the nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) and examined with a Leica microscope equipped for epillumination. In negative control experiments, no immunofluorescence staining was obtained when sections were incubated without the primary antibody, with pre-immune serum as replacement for the primary antibody. The procedure was performed by an investigator blinded to the protocol.

Data analysis

Data are presented as mean ± SD and compared by one-way ANOVA followed by Turkey’s post hoc test for individual significant difference. Electrophysiological data (scoring of programmed electrical stimulation-induced VT/VF) were compared with Chi-squared test. The significant level was assumed at a value of \( P < 0.05 \).
Results

Mortality post-LAD and microinjection

Mortality post-LAD ligation and microinjection was similar among three groups (20% in the PBS, 25% in the MLV and 30% in the SLV group, \( P > 0.05 \)). Mortality after sham operation was zero.

Infarct size, LVEF post-MI and microinjection

One week after infarction and microinjection, the infarct region of the LV was very thin and partly replaced by scar tissue, which was not affected by injection of PBS, MLV or SLV. Infarct size of the SLV, PBS and MLV groups was similar (33.3 ± 2.6%, 31.2 ± 3.5%, 33.8 ± 4.6%, respectively, \( P > 0.05 \), Fig. 1). Pre-LAD, LVEF was about 75% and similar among all groups. Animals with LVEF ≥ 50% at 5 hrs post-LAD were excluded [MLV(2/15), SLV(1/14) and PBS(4/16)]. LVEF was equally reduced among the three MI groups at 1-week after operation (Fig. 2).

QTc interval changes and ventricular vulnerability to VT

The ECG measurements before electrophysiological test of all rats were summarized in Table 3. The QTc interval was significantly shortened in the SLV group compared with the PBS and MLV groups at 1-week post-LAD and microinjection. The incidence of VT by \( S_1S_1 \) stimulus at room temperature during PES was significantly lower in SLV group than in PBS and MLV groups (Fig. 3a). Arrhythmia score was also significantly lower in SLV group than in PBS and MLV groups (Fig. 3b). QTc interval, inducibility of VT and arrhythmias score are significantly higher in PBS and MLV groups than in control group and these parameters are similar between control group and SLV group.

mRNA and protein expression of Sema3a in MI border zone

Reporter gene expression examined by fluorescent microscopy demonstrated lentivirus-derived GFP + labelled host cardiomyocytes at

Fig. 1 Masson’s trichome, blue indicates infarct scar (\( n = 12 \) in PBS group, \( n = 13 \) in MLV group and \( n = 13 \) in SLV group, \( n = 20 \) in CON group).

Fig. 2 Serial analysis of 2D mode echocardiography of CON, PBS, MLV and SLV groups (A). LVEF in the three MI groups was similar at 1 week after infarction and microinjection, \( P > 0.05 \) (B), also the LVDs and LVDd (C and D).
1 week after either MLV or SLV injection, however, GFP+ cardiomyocytes cells were not observed in PBS group and in sham group (Fig. 4). The mRNA-Sema3a level in the myocardium of MI border zone in SLV groups was significantly higher (about twofold) at 1 week after transfection compared with PBS, MLV, CON groups (Fig. 5c). Sema3a protein expression in LV-free wall of sham group was very low and the Sema3a protein expression in the MI border zone from SLV group was significantly higher than that in PBS and MLV group (Fig. 5a and b).

**Nerve sprouting and GAP43 protein expression in MI border zone**

Immunofluorescence staining demonstrated GAP43+ nerve fibres in the MI border zone in three MI groups at 1 week after infarction, and GAP43+ nerve fibres were more abundant in the PBS and MLV groups than in the SLV group (Fig. 6a). Protein expression of GAP43 in MI border zone was significantly higher than that in LV-free wall of sham group, and protein expression of GAP43 in MI border zone was significantly down-regulated in SLV group compared with that in PBS group and MLV group (Fig. 6b and c).

**Sympathetic innervation and protein expression of TH at MI border zone**

The positive TH staining pattern and TH protein expression pattern were similar as changes in GAP43 shown above (Fig. 7).

**Myocardial EPI level in MI border zone**

Although cardiac sympathetic reinnervation was demonstrated by immunofluorescence staining of TH and GAP43, it did not imply that the nerves were also functional. Thus, we determined the circulating and myocardial EPI levels at MI border zone to investigate cardiac sympathetic function. Circulating EPI levels were similar among three MI groups (data not shown). The border zone myocardial EPI levels...

### Table 3 ECG parameters at 1 week after infarction and microinjection

|       | RR(ms) | P(ms) | PR(ms) | QRS(ms) | QTc(ms) |
|-------|--------|-------|--------|---------|---------|
| PBS   | 168.5 ± 10.0 | 16.0 ± 1.2 | 54.9 ± 2.5 | 23.3 ± 1.5 | 178.1 ± 9.5 |
| MLV   | 170.0 ± 7.2  | 16.2 ± 0.7  | 55.0 ± 2.5  | 25.5 ± 1.6  | 180.9 ± 8.2  |
| SLV   | 166.0 ± 7.2  | 16.4 ± 1.6  | 53.4 ± 3.7  | 21.0 ± 1.2  | 168.6 ± 7.8*† |
| CON   | 167.1 ± 5.3  | 16.1 ± 1.6  | 52.5 ± 4.1  | 22.3 ± 1.7  | 165.7 ± 8.3  |

Fig. 3 Incidence of VT and arrhythmia score at 1 week after infarction and microinjection. (A) Incidence of non-self-terminating VTs during PES. (B) Arrhythmia score in the SLV group was significantly lower than that in MLV and PBS groups at 1 week after infarction and microinjection. *P = 0.018, versus the PBS group and †P = 0.025, versus the MLV group respectively.

Fig. 4 Determination of lentivirus transduction and expression in myocardial infarction border zone at 1 week. GFP expression was observed under fluorescence microscopy. (Blue = nuclei, Green = GFP, Red = β-actin, all ×200).
were significantly down-regulated in SLV group compared with PBS or MLV group (Fig. 8).

**Discussion**

In this study, we observed the effect of myocardial overexpression of Sema3a on after infarction sympathetic reinnervation and the inducibility of VT by PES in a rat MI model. The major findings were as follow: (i) Local microinjection of SLV-enhanced protein expression of Sema3a in MI border zone; (ii) Myocardial overexpression of Sema3a-attenuated sympathetic reinnervation in MI border zone and (iii) Myocardial overexpression of Sema3a significantly reduced the incidence of PES-induced malignant arrhythmia in this rat MI model.

It is known that expression of Sema3a mRNA in adult neurons is down-regulated after peripheral nerve injury [17, 27]. Down-regulation of semaphorin expression could result in a more permissive environment for axonal regeneration and sprouting. This study tested the hypothesis that up-regulation of semaphorin expression might attenuate sympathetic hyper-reinnervation post-MI. Our results showed that microinjection of SLV enhanced mRNA and protein expression of Sema3a in the MI border zone and significantly inhibited nerve sprouting and sympathetic hyper-innervation, decreased prolonged QTc intervals and the inducibility of ventricular tachyarrhythmias during EPS in this rat MI model. Our finding suggested that myocardial overexpression of Sema3a post-MI might be beneficial in terms of reducing malignant arrhythmias related to increased post-injury sympathetic nerve density, which is obviously associated with the occurrence of ventricular arrhythmia and SCD in animal models or MI patients [10, 28, 29]. To our best knowledge, this is the first report on the impact of myocardial Sema3a overexpression in this MI model. Consistent with the notion that the nerve sprouting was augmented at the MI border zone [30–32], our results detected excessive sympathetic reinnervation after infarction at the MI border zone, and this process could be inhibited by myocardial Sema3a overexpression in this MI model.
Previous reports showed that MI could induce significant nerve sprouting and sympathetic hyper-innervation, prolonged APD and QTc intervals and increased repolarization dispersion [33]. Cardiac nerve injury caused by MI could also trigger the reexpression of Sema3a and other neurotrophic factor genes in the non-neural cells around the site of injury [34–36], leading to nerve regeneration through nerve sprouting, [9, 37, 38] whereas disturbance on Sema3a expression system was associated with compensatory sprouting and synaptic remodelling [39]. Consistent with above findings observed in the peripheral nervous system, we showed that Sema3a overexpression in rat heart could also inhibit cardiac nerve sprouting and attenuate sympathetic hyper-innervation post-MI in a rat model. The impact of myocardial overexpression on different animal models seemed not to be identical. In a mice transgenic model, overexpression of Sema3a in the heart resulted in both reduced sympathetic innervation and increased susceptibility to ventricular arrhythmias as a result of catecholamine supersensitivity and prolongation of the action potential duration [21]. The underlying mechanisms for the divergent results observed in this rat MI model and the transgenic mice model remains unknown now. It is possible that the myocardial Sema3a overexpression in a post-MI model might help to restore the myocardial Sema3a level to the pre-MI status, thus, be beneficial on inhibiting cardiac nerve sprouting and attenuating sympathetic hyper-innervation post-MI and result in reduced inducibility of malignant arrhythmias during EPS in our model, whereas the general myocardial overexpression of Sema3a in the non-MI transgenic mice model ‘resulted in’ too much myocardial Sema3a which was linked with the observed catecholamine supersensitivity and prolongation of the action potential duration. Further experiments on myocardial Sema3a overexpression in mice MI model are essential to clarify this hypothesis.

Both ventricular arrhythmias and left ventricular dysfunction are related to mortality risk [40]. Previous study show impaired LVEF is a predictor of the presence of ventricular arrhythmias, but the clear relationship between ventricular arrhythmias and left ventricular dysfunction after myocardial infarction is a controversial issue [1]. Our data show no significance among LVEF, LVDs and LVDd of MLV, SLV and PBS group. Thus, the impact of haemodynamic parameters on arrhythmias might be minimal on the observed differences on arrhythmias score and inducibility of VT.

Caution is needed to explore this experimental study results to humans and the impact of increased myocardial Sema3a activity (expression) in MI patients, which could be quite different as we observed in this rat MI model. Myocardial Sema3a overexpression in large animal model might give more hints on potential effects of myocardial Sema3a expression in humans. Nevertheless, our study clearly shows that modulating myocardial Sema3a might be a potential therapeutic option for reducing post-MI malignant arrhythmias and SCD.
Study limitation

It should be mentioned that a chronic study observing the impact of myocardial overexpression of Sema3a on long-term survival, left ventricular remodelling and telemetry monitored 'natural' arrhythmias in this model is essential to conclude the 'general' effect of myocardial overexpression of Sema3a in this model. Moreover, a study exploring the role of down-regulating/inhibiting myocardial Sema3a could highlight the importance of myocardial Sema3a balance post-MI in this model.

Conclusions

Our data show excessive myocardial reinnervation after infarction in this model. This process could be modulated by myocardial overexpression of Sema3a. Myocardial overexpression of Sema3a after infarction can reduce the inducibility of ventricular arrhythmias as a result of attenuated sympathetic reinnervation. This may offer a new potential clinical therapeutic approach for reducing ventricular arrhythmias and SCD post-myocardial infarction.

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Conflict of interest

The authors do not have any financial conflict of interest pertinent to this manuscript.

References

1. Fong MW, Grazeite L, Cesario D, et al. Treatment of ventricular tachycardia in patients with heart failure. Circ Cardiovasc Rep. 2011; 13: 203–9.
2. Myerburg RJ, Castellanos A. Cardiac arrest and sudden cardiac death. In: Braunwald E., editor. Heart disease: A textbook of cardiovascular medicine. 4th ed. Philadelphia, Pa: WB Saunders; 1992. pp. 756–89.
3. The Antiarrhythmics versus Implantable Defibrillators (AVID) investigators. A comparison of antiarrhythmic-drug therapy with implantable defibrillators in patients resuscitated from near-fatal ventricular arrhythmias. N Engl J Med. 1997; 337: 1576–83.
4. Kazmierczak J, Zielonka J, Peregu-Pgorzelnska M, et al. Ventricular and supraventricular arrhythmias and heart failure in a patient with left ventricular noncompaction and Brugada syndrome. Cardiol J. 2011; 18: 310–3.
5. Chen PS, Chen LS, Cao JM, et al. Sympathetic nerve sprouting, electrical remodeling and the mechanisms of sudden cardiac death. Cardiovasc Res. 2001; 50: 409–16.
6. Zhou S, Cao JM, Tebb ZD, et al. Modulation of QT interval by cardiac sympathetic nerve sprouting and the mechanisms of ventricular arrhythmia in a canine model of sudden cardiac death. J Cardiovasc Electrophysiol. 2001; 12: 1068–73.
7. Chang CM, Wu TJ, Zhou S, et al. Nerve sprouting and sympathetic hyperinnervation in a canine model of atrial fibrillation produced by prolonged right atrial pacing. Circulation. 2001; 103: 22–5.
8. Miyachi Y, Zhou S, Okuyama Y, et al. Altered atrial electrical restitution and heterogeneous sympathetic hyperinnervation in hearts with chronic left ventricular myocardial infarction: implications for atrial fibrillation. Circulation. 2003; 108: 360–6.
9. Cao JM, Chen LS, KenKnight BH, et al. Nerve sprouting and sudden cardiac death. Circ Res. 2000; 86: 816–21.
10. Cao JM, Fishein MC, Han JB, et al. Relationship between regional cardiac hyperinnervation and ventricular arrhythmia. Circulation. 2000; 101: 1960–9.
11. Kolodkin AL, Matthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell. 1993; 75: 1389–99.
12. Yu HH, Kolodkin AL. Semaphorin signaling: a little less per-plexin. Neuron. 1999; 22: 11–4.
13. Tamagnone L, Artigiani S, Chen H, et al. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell. 1999; 99: 71–80.
14. Takahashi T, Fournier A, Nakamura F, et al. Plexin-neuropilin-1 complexes form functional neuropilin-3 receptors. Cell. 1999; 99: 59–69.
15. Kolodkin AL, Levengood DV, Rowe EG, et al. Neuropilin is a semaphorin III receptor. Cell. 1997; 90: 753–62.
16. He Z, Tessler-Lavigne M. Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. Cell. 1997; 90: 739–51.
17. Pasterkamp RJ, Giger RJ, Verhaagen J. Regulation of semaphorin III/collapsin-1 gene expression during peripheral nerve regeneration. Exp Neurol. 1998; 153: 313–27.
18. Holtmaat AJ, Gorter JA, De Wit J, et al. Transient downregulation of Sema3A mRNA in a rat model for temporal lobe epilepsy. A novel molecular event potentially contributing to mossy fiber sprouting. Exp Neurol. 2003; 182: 142–50.
19. Ieda M, Kanazawa H, Kimura K, et al. Sema3A maintains normal heart rhythm through sympathetic innervation patterning. Nat Med. 2007; 13: 604–12.
20. Reidy KJ, Villegas G, Teichman J, et al. Sema3A regulates endothelial cell number and podocyte differentiation during glomerular development. Development. 2009; 136: 3979–89.
21. Pfleger A, Kessler T, Silletti S, et al. Suppression of angiogenesis by lentiviral delivery of PEX, a noncatalytic fragment of matrix metalloproteinase-2. Proc Natl Acad Sci USA. 2000; 97: 12227–32.
22. Lee TM, Chou TF, Tsai CH. Effects of pravastatin on cardiomyocyte hypertrophy and ventricular vulnerability in normolipidemic rats after myocardial infarction. J Mol Cell Cardiol. 2003; 35: 1449–59.
23. Nori SL, Gaudino M, Alessandrini F, et al. Immunohistochemical evidence for sympathetic denervation and reinnervation after necrotic injury in rat myocardium. Cell Mol Biol. 1995; 41: 799–807.

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24. Walker MJ, Curtis MJ, Hearse DJ, et al. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. *Cardiovasc Res*. 1988; 22: 447–55.

25. Curtis MJ, Walker MJ. Quantification of arrhythmias using scoring systems: an examination of seven scores in an in vivo model of regional myocardial ischaemia. *Cardiovasc Res*. 1988; 22: 656–65.

26. Gandia C, Arminan A, Garcia-Verdugo JM, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. *Stem Cells*. 2008; 26: 638–45.

27. Hashimoto M, Ino H, Koda M, et al. Regulation of semaphorin 3A expression in neurons of the rat spinal cord and cerebral cortex after transection injury. *Acta Neuropathol*. 2004; 107: 250–6.

28. Friedman DJ, Altman RK, Orencole M, et al. Predictors of sustained ventricular arrhythmias in cardiac resynchronization therapy. *Circ Arrhythm Electrophysiol*. 2012; 5: 762–72.

29. Holzem KM, Eltov IR. Arrhythmogenic remodelling of activation and repolarization in the failing human heart. *Europace*. 2012; 14(Suppl. 5): v50–7.

30. Zhou S, Chen LS, Miyauchi Y, et al. Mechanisms of cardiac nerve sprouting after myocardial infarction in dogs. *Circ Res*. 2004; 95: 76–83.

31. Verrier RL, Kwaku KF. Frayed nerves in myocardial infarction: the importance of rewiring. *Circ Res*. 2004; 95: 5–6.

32. Adlan AM, Lip GY, Fadel PJ, et al. Sym pathetic nerve activity during non-sustained ventricular tachycardia in chronic heart failure. *Int J Cardiol*. 2012; 22: 01521–5.

33. Himura Y, Felten SY, Kashiki M, et al. Cardiac noradrenergic nerve terminal abnormalities in dogs with experimental congestive heart failure. *Circulation*. 1993; 88: 1299–309.

34. Levi-Montalcini R. Growth control of nerve cells by a protein factor and its antisera: discovery of this factor may provide new leads to understanding of some neurogenetic processes. *Science*. 1964; 143: 105–10.

35. Lindholm D, Heumann R, Meyer M, et al. Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. *Nature*. 1987; 330: 658–9.

36. Rush RA. Immunohistochemical localization of endogenous nerve growth factor. *Nature*. 1984; 312: 364–7.

37. Guth L. Regeneration in the mammalian peripheral nervous system. *Physiol Rev*. 1956; 36: 441–78.

38. Vracko R, Thoring D, Frederikson RG. Fate of nerve fibers in necrotic, healing, and healed rat myocardium. *Lab Invest*. 1990; 63: 490–501.

39. Pasterkamp RJ, Anderson PN, Verhaagen J. Peripheral nerve injury fails to induce growth of lesioned ascending dorsal column axons into spinal cord scar tissue expressing the axon repellent Semaphorin3A. *Eur J Neurosci*. 2001; 13: 457–71.

40. Bigger JT Jr, Fleiss JL, Kleiger R, et al. The relationships among ventricular arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. *Circulation*. 1984; 69: 250–8.