Modeling of the cardiac sympathetic nervous system and the contribution of epicardium-derived cells

Ge, Y.

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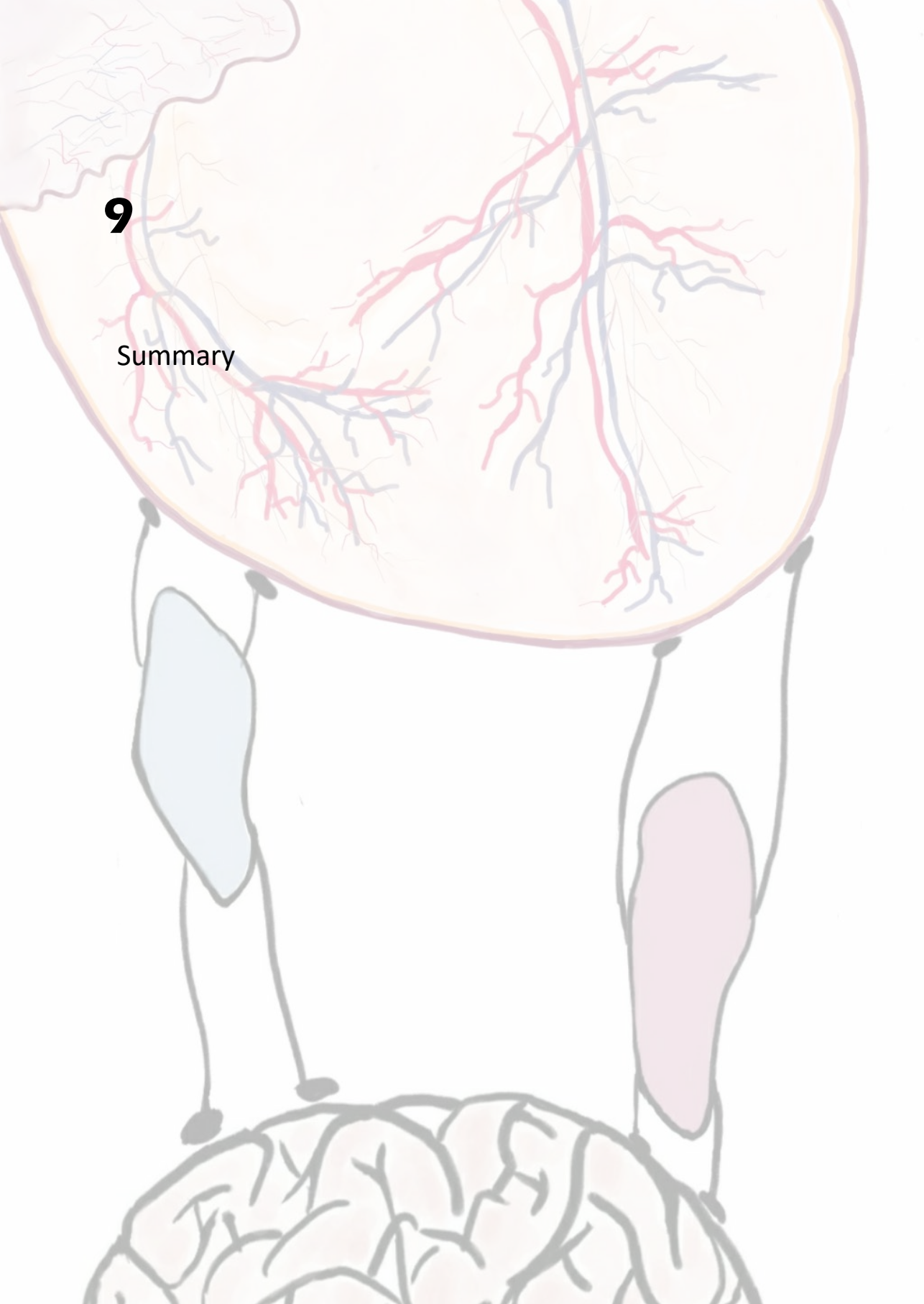
Summary



Summary

About one third of all global deaths are attributed to cardiovascular diseases (World Health Organization 2017). Sudden cardiac death (SCD) is a worldwide public health challenge and is commonly associated with ischemic heart disease. SCD after myocardial infarction (MI) is classically attributed to heterogeneous conduction in the infarct border zone. However autonomic hyperinnervation (especially sympathetic hyperinnervation) after MI has also been related to sudden cardiac death in a myriad of reports. The heart is innervated by the autonomic nervous system, which can be divided into sympathetic and parasympathetic parts. The balance between cardiac sympathetic and parasympathetic innervation is critical for maintaining normal cardiac function. Excessive sympathetic activation can be pro-arrhythmogenic. Post-MI hyperinnervation is most often linked to an increase of sympathetic nerve sprouting, which has been discovered in both patients and animal models. This phenomenon of post-MI sympathetic hyperinnervation has raised growing awareness on the important role of pathological innervation patterns in arrhythmogenesis, however the mechanism is not clear yet.

In **Chapter 1**, a general introduction to this thesis is provided. The heart is a self-excitation organ and is composed of three layers, from outermost to innermost the epicardium, myocardium and endocardium are encountered. Although the outer layer, the epicardium is a thin layer of cells, it plays an essential role in cardiac development. During development, epicardium-derived cells (EPDCs) can migrate into the subepicardial space and differentiate into several cardiac cell types. In addition, they have the ability to influence myocardial growth and coronary vessel patterning via paracrine effects. The cardiac autonomic nervous system, another critical element of a functional heart, regulates heart rate, contraction force and atrioventricular conduction velocity to adjust the body's adaption to different conditions. In this chapter, the development of epicardium and cardiac autonomic nervous system is introduced, as well as their dynamic changes in cardiac diseases, such as MI.

In **Chapter 2**, the role of EPDCs in promoting cardiac sympathetic re-/hyperinnervation after cardiac damage is elucidated *in vitro*. Sympathetic ganglia were co-cultured with mesenchymal EPDCs and/or myocardium, and neurite outgrowth and sprouting density were assessed. A significant increase in neurite density and directional (i.e. towards myocardium) outgrowth was observed when ganglia were co-cultured with a combination of EPDCs and myocardium, as compared to cultures with EPDCs or myocardium alone. The promotional effect of mesenchymal EPDCs on sympathetic neurite sprouting via paracrine signaling was confirmed by culturing PC12 cells in conditioned EPDC-medium, and a role of NGF, Endothelin-1 and SEMA3A in the process was also demonstrated in this study.

In **Chapter 3**, the influence of the sex of the donor that EPDCs are derived from on cardiac sympathetic innervation was studied. Co-cultures were performed of sympathetic ganglia with myocardium and activated EPDCs. Combinations of both male and female cellular components were included in the co-cultures to elucidate the impact of sex on cardiac sympathetic re-/hyperinnervation *in vitro*. The key findings include: i) EPDCs promote sympathetic neurite outgrowth in vitro and increase the directional neurite projection towards myocardium; ii) In the presence of EPDCs, male sympathetic ganglia exhibit higher cardiac sympathetic outgrowth than female ganglia; iii) Male EPDCs in a female setting can increase the cardiac sympathetic neurite outgrowth to a level that is comparable to the level of outgrowth in an entirely male environment. We propose the Slit2/ROBO-pathway as a potential candidate influencing these differential findings in male and female EPDCs. The findings in this chapter underline the potential relevance of sex differences in post-MI cardiac hyperinnervation. These data also suggest that sex should be taken into account when considering injection of cells for cell therapy in male and female patients.

In **Chapter 4**, an polyclonal line of inducible proliferative human EPDCs (iEPDCs) was established to facilitate in-vitro study using EPDCs, such as exploring the mechanism of their impact on cardiac sympathetic outgrowth. Inducible proliferation was achieved by doxycycline-controlled expression of simian virus 40 large T antigen (LT) with a repressor-based lentiviral Tet-On system. After doxycycline removal, LT expression ceased and the iEPDCs regained their cuboidal epithelial morphology and could undergo epithelial-to-mesenchymal transition (EMT) after stimulation with transforming growth factor β3, similar as primary EPDCs. This was confirmed by RT-qPCR analysis and (immune)cytochemical staining. Collagen gel-based cell invasion assays demonstrated that mesenchymal iEPDCs, like primary EPDCs, possess increased invasion and migration capacities as compared to their epithelial counterparts. In addition, mesenchymal iEPDCs stimulated sympathetic neurite outgrowth similarly to primary EPDCs. This renders iEPDCs a highly useful new model for studying human epicardial properties in vitro.

Post-MI sympathetic hyperinnervation is most often linked to an increase of sympathetic nerve sprouting from cardiac sympathetic ganglia. In **Chapter 5**, the remodeling process of cardiac sympathetic ganglia after MI was explored. The superior cervical ganglia (SCG), situated adjacent to the carotid body (CB), contribute to cardiac ventricular sympathetic innervation. Remodeling of SCG at 24 hours, 3 days, 7 days and 6 weeks after MI was investigated with histological evaluation. SCG remodeling was observed as early as 24 hours after infarction, with a peak at day 7, regressing within 6 weeks post-MI to basal levels with a significant increase in neuron size and a decreased intensity of ChAT expression. Moreover, the most robust neuronal remodeling was observed at the region adjacent to the CB with an

increase of neurotrophic factors. Furthermore, the high affinity receptors of BDNF and NGF increased in the SCG after MI. We concluded that overt remodeling occurs in the SCG as well as in the CB, suggesting an interaction of these 2 structures after MI, that might contribute to pathological cardiac hyperinnervation.

Chapter 6 describes a detailed low-input sample preparation method for single-nucleus sequencing, which includes the dissection of mouse superior cervical and stellate ganglia, cell dissociation, cryo-preservation, nucleus isolation and hashtag barcoding. The method has the potential for broad application also in studies of innervation of other organs and tissues.

Chapter 7 applies the method described in Chapter 6 to explore SCG remodeling after MI with single nucleus RNA sequencing (snRNA-seq) technology. snRNA-seq of healthy murine SCGs was performed, creating a template of the cellular composition of the SCG. The molecular signatures of neuronal and satellite glial cells at healthy baseline were identified, which will be utilized for investigation of disease models in future studies.

Chapter 8 provides a systematic review of current morphological evidence on the contribution of the SCG to cardiac innervation in health and disease in human and other animal models. A general discussion in relation to the work presented in this thesis, is also included in this chapter.