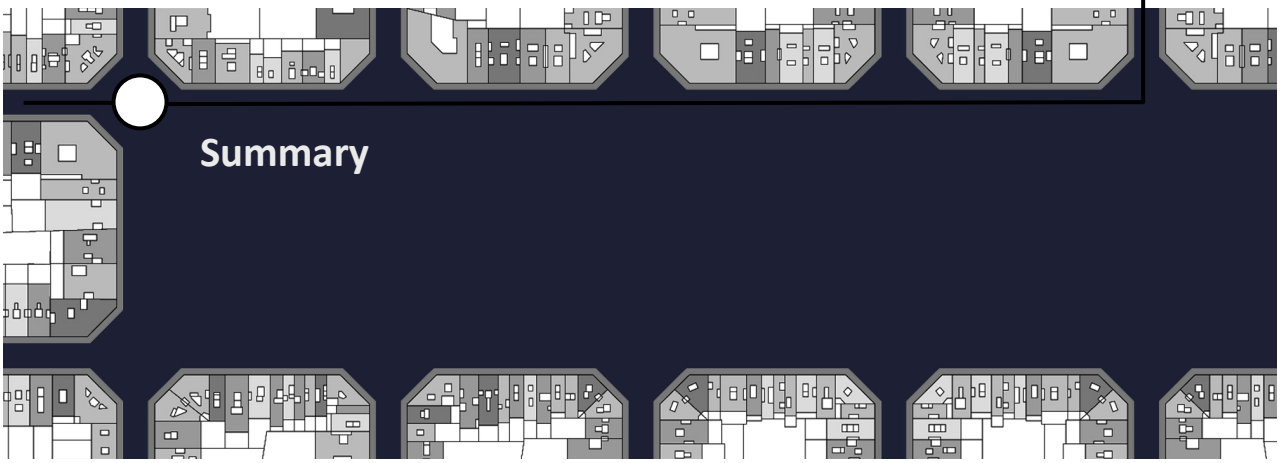
Development of the sinus venosus myocardium from the posterior second heart field : implications for sinoatrial and atrioventricular mode development

Vicente Steijn, R.


Summary

Summary

In this thesis we have described the cellular contribution of second heart field-derived cells to the developing heart, focusing on the morphological and functional development of the sinus venosus myocardium and components of the central cardiac conduction system (CCS), specifically the sinoatrial (SAN) and the atrioventricular (AVN) node. For this, we have studied the incorporation of cells from the posterior heart field (a subpopulation within the second heart field) to the venous pole of the heart in mouse, chicken and quail models using several immunohistochemical and molecular markers like *podoplanin*, *RhoA*, *PDGF*-family, *Id2* and *Hcn4*. We have also studied the changes in the embryonic electrogram in relation to potential arrhythmogenic substrates that could be explained by events during development.

In **chapter 1** we provided an overview on heart development and the development of the CCS. These two processes are closely related and abnormal development of the heart can result in abnormal CCS development. Within cardiac development, the recruitment of progenitor cells from the first and second heart field are essential. We have focused on the progenitor cells that will contribute to the venous pole of the heart (posterior heart field) both to a myocardial and a mesenchymal component, from which a partial contribution to the CCS is proposed. The composition and development of two major structures within the CCS, the SAN and AVN was reviewed.

In **chapter 2** we described the role of the transmembrane glycoprotein podoplanin in posterior heart field development. We investigated the role of *podoplanin* in development of the sinus venosus myocardium. *Podoplanin* mutants showed a generalized hypoplasia (underdevelopment) of the myocardial structures at the venous pole of the heart. The sinus venosus myocardium including the SAN, the myocardium lining the wall of the cardinal and pulmonary veins, the primary atrial septum and dorsal atrial wall were all hypoplastic in the *podoplanin* mutant embryos. Impaired myocardial formation was correlated with abnormal epithelial-to-mesenchymal transformation of the coelomic epithelium due to upregulated E-cadherin and downregulated RhoA, which are controlled by *podoplanin*. Our results demonstrate an important role for *podoplanin* in the development of the sinus venosus myocardium.

Chapter 3 further focused on the development of sinus venosus myocardium including the SAN in the chick by studying expression patterns of RhoA in relation to other markers, and by studying electrical activation patterns of the developing sinus venosus myocardium. During development, initial myocardium-wide expression of RhoA became restricted to the right-sided sinus venosus myocardium, comprising the SAN. Electrophysiological measurements revealed initial capacity of both atria to show the first electrical activity that in time shifted to a right-sided dominance, coinciding

with persistence of RhoA, Tbx18 and *Hcn4* and absence of Nkx2.5 expression in the definitive SAN. The remaining sinus venosus myocardium further differentiates towards a 'working myocardium phenotype'. We hypothesize an initial sinus venosus wide capacity to generate pacemaker signals, becoming confined to the definitive SAN. Lack of differentiation towards a chamber phenotype could explain ectopic pacemaker foci.

In **chapter 4** we described the expression patterns of PDGF-A, -C and PDFGR-α during chick heart development. The expression patterns observed were spatiotemporally associated with the development of the second heart field at the arterial and venous pole of the heart and the development of the proepicardial organ and its derivatives, the epicardium and the epicardium-derived cells (EPDCs). Inhibition of epicardial outgrowth resulted in abnormal expression of both ligands (PDGF-A, -C) and the receptor (PDFGR-α), whereas pharmacological inhibition of PDGFR-α signalling resulted in abnormal epicardial development. These findings suggest that PDGF-A, -C and PDGFR-α are involved in cardiac remodelling during development, specifically related to the ventricular compact and trabecular myocardium through epicardial-myocardial interactions.

In **chapter 5** we described the expression of the inhibitor of differentiation *Id2* during heart development in relation to the second heart field. A possible function for *Id2* in distal CCS patterning has been suggested. Id2 had a broad expression pattern in the heart and vascular system and was expressed in myocardial progenitor cells at both the inflow and outflow tract of the heart, as well as in cells of the endocardial, epicardial and neural crest cell lineage. It was not expressed in fully differentiated myocardial cells. *Id2* knockout embryos demonstrated severe cardiac defects that include abnormal orientation of the systemic and pulmonary drainage, abnormal myocardialization of systemic and pulmonary veins, hypoplasia of the SAN, atrial and ventricular septal defects and myocardial hypoplasia, and double outlet right ventricle. These abnormalities suggest a role for *Id2* in second heart field deployment.

In **chapter 6** we have studied expression patterns of the cation channel HCN4 during chick CCS development. In situ hybridisation for *Hcn4* in the chick had not been performed yet due to the lack of the sequence in the databases. After obtaining the full-length sequence for *Hcn4, w*e designed our own RNA probe to detect *Hcn4* during chick heart development. Expression was observed early in development in the primary heart tube. After looping of the heart had started, expression became restricted to transitional zones flanked by working myocardium, i.e. myocardium that will not contribute to the CCS. These transitional zones include the sinus venosus myocardium where the SAN develops, the atrioventricular canal myocardium, the primary fold (a myocardial zone between the developing ventricles), and the developing outflow tract. Further in development, *Hcn4* expression was restricted to

the SAN, the AVN, the common bundle, the bundle branches and the internodal and atrioventricular ring myocardium. Based on these results we have identified *Hcn4* as a marker of the developing CCS in the chick. Furthermore, we propose that expression patterns during development can explain the occurrence of arrhythmogenic anatomical predilection sites in adults.

Chapter 7 provided an overview of the morphological changes in SAN and AVN differentiation during avian heart development. We also provided baseline measurements of developmental changes in the electrogram as well as the results of our lineage tracing experiments. Initially, the entire sinus venosus myocardium shows a specific panel of markers and has the potential to generate the first electrical activity. At later stages both expression patterns and electrical activation patterns become restricted to the definitive right-sided SAN. Similarly, the restriction of expression pattern for specific cardiac markers is also observed in the atrioventricular canal myocardium, where the future AVN will develop and initially cannot be distinguished from the surrounding myocardium. Lineage tracing experiments present a potential sinoatrial contribution to the AVN area. On an electrophysiological level, we observed a significant increase in heart rate and atrioventricular delay during development. These results show that significant changes occur in both morphology and electrical properties of chick sinus venosus and atrioventricular ring myocardium during development, where the putative SAN and AVN will form, respectively. The broad electrical potential of these structures during development may form an explanation for the occurrence of predilection sites for arrhythmias in the adult.

Finally, **chapter 8** provides a general discussion on the data presented on this thesis. The development of the heart in relation to the formation of the CCS is discussed with focus on understanding the developmental processes that could account for potential arrhythmogenic substrates. We have provided evidence (**chapters 1, 3, 6** and**7**) that the embryonic area of the developing CCS is broader than what is found in the adult. Potentially, embryonic structures could retain or re-gain this potential which could lead to rhythm disturbances. The next necessary step involves thorough lineage tracing experiments to study the cellular composition of the pertinent CCS structures like the SAN or the AVN.