The myocardial potential of proepicardial cells: From development to cardiac regeneration
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Scope

Cardiovascular diseases, in particular ischemic heart disease, are the leading cause of death in the western world. Cardiomyocytes that are lost upon ischemia are replaced by fibrotic scar tissue. This, in turn, results in a diminished cardiac pumping capacity and serious morbidity of the patient. Despite available cardiac intervention treatments such as pharmacotherapeutics or stent assisted revascularization and cardiac surgery, the infarcted part of the heart will never fully recover. In this respect, it can be envisioned that insights into the mechanisms underlying cardiac muscle cell formation during development, could be very instrumental in the search for new treatments for this group of patients. During development, progenitor cells differentiate into the working myocardium of the atria and ventricles, as well as myocardial cells of the conduction system. The coronary vasculature and non-myocardial cell population, which together make up 70% of the total of adult cardiac cells, are added to the heart during its development. Knowledge of these processes should provide a solid base required to develop new strategies to treat patients suffering from the loss of cardiomyocytes by ischemic damage.

Development of the heart

The heart is the first organ formed during embryogenesis. At 3 weeks of development in humans, which corresponds to 8.5 days of development in mice or 2 days of chicken development, the heart starts to contract. The heart initially develops from mesoderm of the coelomic wall, as a simple muscular tube that propels the blood in a peristaltic fashion. As development proceeds, the heart tube loops and the atria and ventricles begin to balloon out at the outer curvatures of the now “S-shaped” tube. Endocardial cushions are formed at specific locations of the heart, namely the atrioventricular canal and outflow tract. Such cushions will give rise to the mature valves and contribute to the septa. At this stage the heart is not covered by epicardium as is the case for the adult heart. The early embryonic heart lies, so to speak, “naked” within the pericardial cavity. During development the epicardium develops from the proepicardium, a small, transient structure which is formed at the inflow pole of the heart. Proepicardial cells attach to, and spread over the myocardium of the heart tube, forming the embryonic epicardium. The embryonic epicardium further differentiates into the adult epicardium, and contributes to the coronary vasculature, cardiac fibroblast population, and valves. By septation, valve formation, differentiation of working and conduction myocardium, and epicardium formation the primitive heart has transformed into the adult four-chambered heart.

In the adult heart, oxygen-poor blood from the body, enters the right atrium via the superior and inferior caval veins (figure 1a). Via the right atrium the blood enters the right ventricle and is pumped into the pulmonary artery towards the lungs where it becomes oxygenated. The blood then returns to the heart via pulmonary veins and the left atrium
and travels to the left ventricle from where it is pumped into the aorta. The aorta is the main systemic artery of the body from which all other arteries branch off, providing the body with oxygenated blood. The myocardium of the heart is oxygenated via the coronary arteries which branch off from the aorta directly above the aortic valves. Within the chest, the heart is located within the pericardial cavity. This cavity is lined by the pericardium, a loose connective tissue covered by a mesothelial layer producing a mucous fluid which facilitates smooth cardiac movement (Figure 1b). The connective tissue of the pericardium becomes fibrous, giving strength to the pericardial sac. The mesothelium covering the heart, is called the epicardium.

**Focus of this study**
This thesis focuses on the mechanisms of development of the epicardium and the role of the epicardium in response to ischemic damage in the adult heart. Using in vitro tissue culture we have studied the effect of several growth factors, proteins that function as signaling molecules between cells, on the differentiation of proepicardial cells. The regulation of proepicardial differentiation was further assessed by micro-array analysis. The role of the epicardium in response to injury was analyzed in a Left Anterior Descendent (LAD) coronary artery ligation model in wild type and genetically modified mice that allow the analysis of the fate of epicardial cells using the Cre-loxP system.

In chapter 1, the options for cardiac regeneration are discussed from a developmental point of view. Chapter 2 reviews the role of Bone Morphogenetic Proteins growth factors in cardiac development. In chapter 3 we describe the analysis of the myocardial
potential of the proepicardium and identify growth factors important in the regulation of the differentiation of proepicardial cells, i.e Bone Morphogenetic Protein and Fibroblast Growth Factor. The common progenitor pool of myocardial and proepicardial cells, and the molecular mechanism of the interaction of Bone Morphogenetic Protein and Fibroblast Growth Factor signaling pathways are further substantiated in chapter 4. The results of this study, in relation to recently published findings on this topic, are reviewed and discussed in chapter 5. A genome-wide search for genes that play a role in proepicardial differentiation is described in chapter 6, in which we identified the extracellular modulator of Wnt-signaling, Wnt-inhibitory factor 1, as a stimulator of myocardial differentiation of proepicardial cells. Finally, in chapter 7, the role of the adult epicardium in response to injury is investigated in a LAD-ligation model in mice. This analysis shows that a regenerative response, comparable to the response observed in fish, is also initiated in the mouse. This reveals that an evolutionary conserved mechanism also appears to persist in the mammalian heart. However, unlike in fish, the regenerative response of the higher vertebrate, mammalian heart is abrogated, resulting in the formation of only a limited number of new cardiomyocytes.

Taken together, these findings provide a level of basic knowledge which could be useful for the development of new strategies to enhance the endogenous regenerative response of the myocardium and eventually heal the infarcted heart.