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Institutionen för medicin Huddinge, enheten för kardiologi

Embryonic and Adult Cardiac Stem cells- Molecular, Electrophysiological and Immunological Characteristics for Cardiac Repair

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Abstract

Background: The concept of myocardial regeneration by means of stimulating the endogenous regenerative potential *in situ* is an attractive approach. This offers distinct advantages to stem cell implantation where the problems with engraftment and immune rejection are avoided. Resident cardiac progenitors (CPCs) have emerged as promising optimal candidates for cardiac repair. The overall aim of this thesis was to identify and characterize the Isl1+ cells as promising CPCs; from the molecular, electrophysiological as well as immunological aspects in which human embryonic stem cells (HESCs) were used as a template for our planned transplantation studies.

Methods and Results: In *papers I-III*, based on both protein and transcriptional level analyses, we have identified the Isl1+ CPCs throughout the entire life span, both from the embryonic human heart and from the embryonic to the adult rat heart. Early in development the Isl1+ cells were mainly in the para-cardiac regions (pharyngeal foregut endoderm, splanchnic mesoderm, areas suggested to be the second heart field), while later in development they become predominantly localized in the following cardiac subdomains: outflow tract, inflow region of the right atrium and the upper part of right ventricle. Some of the Isl1+ cells were differentiating, while others were undifferentiated. However, only a minority of the Isl1+ cells was proliferating in contrary to the majority of the ventricular embryonic cardiomyocytes. After birth, immature Isl1+ cells were still present in the OFT where they resided until adulthood. Their distribution within the heart matched the defined embryonic distributions. Spontaneously beating *in vitro* cardiospheres were obtained from the embryonic human heart, exhibiting rate-response to electrical and pharmacological stimuli. To explore how cardiac regeneration and cell turnover adapted to disease, different forms of stress; physiological and pathological were studied for their effects on the CPC markers c-Kit and Isl1. Among the different stress modalities, ischemia-reperfusion (IR) injury was the strongest stimulus for activation of markers suggesting endogenous cardiomyocyte regeneration, correlating to the endogenous up-regulation of IGF-1 and HGF. There was a spatial mismatch on one hand of c-Kit and on the other hand Isl1 expression.
In *paper IV*, HESCs were used to test if the triple costimulation blockade regimen in a mouse model could induce a long-term immune tolerance to *in-vivo* transplanted HESCs to the testis and the heart. Costimulation blockade induced tolerance to undifferentiated HESCs in the immune-privileged environment of the testis and induced regulatory T-cells to HESCs when transplanted into the myocardium of immunocompetent mice. A booster dose of costimulation blockade induced HESC engraftment in one out of five immunocompetent mice.

Conclusions: The human embryonic heart is a potential source for the Isl1+ CPCs. These Isl1+ CPCs are present during the whole life span from the embryonic period until adulthood. They seem to have the capacity to differentiate into a cardiac specific-lineage. IR injury among other stresses was the strongest stimulus with both global and focal cardiomyocyte progenitor cell markers up-regulations in the adult heart. Short-term treatment with the costimulation blockade is sufficiently robust to induce long-term tolerance to transplanted HESCs in an immune-privileged environment and to induce regulatory T-cells when transplanted to the myocardium.

Key words: human resident cardiac progenitor, Isl1, c-Kit, Nkx2.5, IGF-1, HGF, ischemia-reperfusion injury, HESCs, costimulation blockade and tolerance.

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