State of the heart : the promise of pluripotent stem cell-derived cardiomyocytes in disease modelling, differentiation and development

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CHAPTER 8

English summary

Pluripotent stem cells (PSCs) can multiply indefinitely in culture and differentiate into all specialized somatic cells of the adult body. We make use of embryonic stem cells (ESCs), isolated from the pre-implantation embryos, and induced pluripotent stem cells (iPSCs), generated by reprogramming somatic cells to understand human cardiac lineage development and disease.

Chapter 1 introduces the pluripotent stem cell field historically from the first derivation of ESCs and how to maintain these cells in a pluripotent state in vitro. The generation of iPSCs revolutionized the stem cell field in 2007 because it allowed pluripotent stem cells to be derived from individuals with diseases, so that if the disease had a genetic origin the cells could provide a model for it. Both types of pluripotent stem cells can differentiate into cells from all three germ layers of the developing foetus, including endothelial cells and cardiomyocytes of the cardiovascular system. The review describes cardiac differentiation protocols and how they mimic early cardiac development in the embryo. Aside from using these cells for modelling genetic diseases, we conclude that we can also use these cells for studying development as well as for drug and toxicity testing and possibly regenerative medicine.

Chapter 2 continues to evaluate the possible applications of PSC-derived cardiomyocytes in the context of studying cardiac diseases. These cells give the unique opportunity to generate human cardiomyocytes in vitro from both healthy individuals and from patients. We provide examples of several cardiac diseases, such as ion channelopathies and cardiomyopathies and the potential of PSCs in their study. In addition, we expound the use of PSCs in cardiac drug discovery and development, and the challenges for future research.

To investigate the possibility of studying inherited cardiac diseases using pluripotent stem cells, we compared how similar PSC-derived cardiomyocytes were to cardiomyocytes isolated from the heart itself. **Chapter 3** describes research on a complex cardiac overlap syndrome caused by a mutation (1795insD) in *SCN5A* – a gene encoding a sodium ion channel that causes both a gain- and loss-of-function genetic disorder. Patients with the mutation can display symptoms of both long-QT syndrome type 3 and Brugada syndrome. First, we generated iPSCs from a mouse with the analogous *SCN5A* mutation. This mutation had also previously been introduced into mouse ESCs. We were able to show that cardiomyocytes from both the iPSCs and ESCs displayed similar altered electrophysiological properties as primary cardiomyocytes isolated from adult heterozygous mice with the same mutation. We then derived human iPSCs from a patient with the *SCN5A-1795insD* mutation. The cardiomyocytes derived from these human cells showed changes in the biophysical properties similar to those observed in all three types of mouse cardiomyocytes. We also demonstrated that both ESC- and iPSC-

derived cardiomyocytes can recapitulate the characteristics of a complex overlap syndrome, despite of the electrophysiological immaturity of PSC-derived cardiomyocytes.

The research next focused on refining the techniques to differentiate the human PSCs to cardiomyocytes. **Chapter 4** contains a detailed description of a directed cardiac differentiation protocol for pluripotent stem cells. We developed a monolayer differentiation procedure to generate cultures comprising of ~50 % cardiomyocytes. We use defined media that is effective for maintaining various pluripotent stem cell cultures in an undifferentiated state as a starting point. This was the only protocol that supported the growth and differentiation of several hESC and hiPSC lines under identical conditions. Furthermore we outlined protocols to quantify and evaluate the efficiency of differentiation.

These protocols were applied in the study comparing hESC- and hiPSC- derived cardiomyocytes directly at different time points during cardiac differentiation that is described in **chapter 5**. The fluorescent marker GFP was targeted to one allele of *NKX2-5* in a hiPSC line that now matched a similar hESC reporter line previously generated in the laboratory. This offered the opportunity to obtain cardiomyocytes and their precursors at different time points during the differentiation and determine the true degree of similarity between both PSC sources since both lines were maintained and differentiated under identical defined conditions. We found that comparable cardiac populations derived from both ESCs and iPSCs could be isolated. Gene expression profiling comparing these cell lines during differentiation indicated that hiPSCs are very similar to hESCs and are a useful surrogate to study early cardiac development and serve as a replacement for cardiomyocytes derived from hESCs.

The same hESC- and hiPSC-derived cardiomyocytes were also compared to a unique set of fetal heart samples. **Chapter 6** focuses on the gene expression profiles of matched atria and ventricles from several time points during the first and second trimester of development and these were compared to hESC- and hiPSC-derived cardiomyocytes. We found that PSC-derived cardiomyocytes most closely matched the first trimester heart samples, but when PSC-derived cardiomyocytes are cultured in a medium containing thyroid hormone they could mature and were more closely related to the second trimester samples.

Finally, in **chapter 7** the results of the studies are summarized and discussed in the light of implications for future research.