Fluorescent gene reporters in human pluripotent stem cells : as model for studying human heart development and cardiomyocyte differentiation

Hartogh, S.C. den

SUMMARY

The establishment of human pluripotent stem cell (hPSC) technology and advanced cardiomyocyte differentiation protocols has opened a new platform for regenerative medicine, cardiac development, and assay development for tissue engineering, disease modelling, and drug toxicity testing. The need for these assays will be largely increasing in our nearby future, due to strengthened legislations, aiming at decreasing adverse drug reactions, and reducing the use of animal research in drug efficacy and risk management studies. Moreover, since the ability to generate patient- and disease- specific hiPSC lines, our health care system will slowly move towards the idea of personalized medicine, a model that aims at patient-specific medical treatments, based on genetic and biomarker information. Such a model can both lower costs and increase the quality of health care, and can therefore be of high importance for a sustainable health care system in our future.

In this PhD research

For pharmaceutical applications, it is important that cardiomyocyte-based assays are of highly predictive value. The use of cardiomyocyte subtypes in such assays could be of great importance, which may include atrial or ventricular cardiomyocytes, or cells of the cardiac conduction system. Moreover, the combination of different cardiomyocyte subtypes could also play a role in successful cellular transplantation therapies of the damaged heart. Therefore, it is of great interest to develop differentiation protocols that direct towards the generation of specific subtypes of cardiomyocytes. In this thesis, we describe the generation of fluorescent stem cell reporter lines to obtain insights into molecular mechanisms that play role in the formation of early cardiac progenitors and their commitment to cardiomyocyte subtypes.

A comprehensive overview of existing fluorescent reporter lines and their developmental and translational applicability is given in **Chapter 2**. Further, we describe in **Chapter 3** how the generation of a dual cardiac reporter MESP1$^{mCherry/w}$ NKX2-5$^{eGFP/w}$ hESC line allows us to study the formation of MESP1 expressing pre-cardiac mesoderm progenitors and their further commitment towards the cardiac lineage, in which key cardiac transcription factor NKX2-5 becomes expressed. We show the characteristics of MESP1-expressing progenitors and their cardiac derivatives based on gene expression profiling and surface marker expression analysis **(Chapter 3,4)**. In **Chapter 4**, we point towards putative cardiac (co)-regulators, based on their temporal expression profile upon cardiac differentiation, and their predicted protein-protein interaction with established key cardiac transcription factors. Functional experiments are required to identify their definite role.

In Chapter 5 and 6 we describe the generation of dual cardiac reporters TBX3e-mCherry-NKX2-5$^{eGFP/w}$ and MYL2-T2A-mCherry-NKX2-5$^{eGFP/w}$ hESC lines, aiming at obtaining either human nodal-like or ventricular cardiomyocytes *in vitro*, respectively. We discuss the technical and biological hurdles that we faced upon developing directed differentiaton

protcols towards nodal-like cardiomyocytes. Further, we showed how MYL2 expression levels in early ventricular cardiomyocytes were low, and did only increase after long-term culture. Interestingly, we showed how MYL2 levels could be induced upon culture in maturation-inducing medium, which could indicate MYL2 as potential cardiomyocyte maturation marker.

Further, in **Chapter 7**, we showed how proliferation reporter Anillin, fused to fluorescent protein eGFP, could be used to visualize distinct phases of the cell cyle, including cell division. We indicate how the visualization of proliferation using such a marker, in combination with automated quantification software, could be of potential use for high-throughput screenings of molecules playing a role in cardiac progenitor development and/or cardiomyocyte regeneration.

In conclusion **(Chapter 8)**, the innovation of advanced molecular technologies and efficient directed cardiomyocyte differentiation protocols will contribute to a better understanding of the complexicity of how epigenetics and transcriptional networks play a crucial role in cardiac lineage commitment and the onset of disease. In-depth knowledge into cardiomyocyte differentiation and development is a prerequisite for translational applications of cardiomyocytes, such as cellular therapy, or drug discovery/safety screening assays.