Cardiomyocytes from human induced pluripotent stem cells: capturing disease severity of LQT2 syndrome and the impact of chromosome aberrations

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SUMMARY

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Complexity is not necessarily expected from monogenic diseases but for many cardiovascular diseases (CVD), simple genotype-phenotype relationship may be far from reality. As millions of people globally die of CVD, it is important to find models to study CVD that recapitulate the conditions as manifest in humans, most importantly for these cases of unexpected complexity. For these, simple gene mutation or deletion in mice has often failed. Combining human induced pluripotent stem cell (hiPSC) with genetic editing technologies is providing new opportunities to bridge the gap, with many hPSC-CM models now showing promising results for testing drugs, discovering molecular pathways associated with disease and other types of (gene) therapies. The work in this thesis contributes to this area of research.

Chapter 1 introduces and reviews the principal features of human pluripotent stem cells (hPSCs) and their contributions to date on the development of the cardiac disease modelling field. The ability to generate hiPSCs from patients with CVD and then differentiate them into cardiomyocytes has allowed researchers to investigate the molecular mechanisms of CVD and speculate about the effects of drugs on the heart. The chapter ends with discussion on how genetic instability is a potential challenge in the generation and maintenance of hiPSC since chromosomal aberrations, copy number variations (CNVs) and point mutations can all develop during passage in culture.

In **Chapter 2**, a comprehensive review illustrates the current state of the field regarding the generation of cardiomyocytes from hPSCs, methods to assess them functionally and a detailed overview of current hPSC cardiac disease models. We evaluate whether treatments indicated by these *in vitro* models could actually be translated to clinical practice and consider current shortcomings and future aspects of this model.

In **Chapter 3**, we establish a robust method for generation and characterisation of genetically modified hiPSCs using Crispr/Cas9 technology, focusing on introduction of heterozygous point mutations which could be used for the creation of LQT2 models.

Chapter 4 and 5 show the applicability of genetically edited isogenic hiPSC-CM lines to model the disease severity of LQT2 *in vitro*, and the effect of a common genetic variant (KCNH2-K897T) postulated to alter disease manifestation.

In **Chapter 6**, we evaluate the molecular impact of complete loss of wild-type hERG channels in hiPSC-CMs in the heterozygous *KCNH2* mutant hiPSC lines described in the previous chapters. We identify that such compound mutations led to the activation of the ubiquitination-proteosome pathway and to the modification on gene expression of certain ion channels.

Chapter 7 examines the genetic stability following cell culture maintenance and passage. We establish the importance of a correct cell line characterization upon cell reprogramming or subcloning. As a first study in the field, we show the impact of a common duplication within chromosome 1 on hiPSC-CM contractility, discussing the influence of these abnormalities to develop disease models.

Finally, **Chapter 8**, gives a general discussion of the studies performed and presented in this thesis and summarizes hypothesis, limitations and hope for improvement on risk stratification, precision medicine approaches and drug development.