Using human pluripotent stem cell-derived cardiomyocytes to understand genetic variant pathogenicity in the ion channelopathy LQT2

Brink, A.H. van den

Summary

One of the main challenges in the clinical management of patients with monogenic cardiac disease patients is the clinical heterogeneity in disease severity. While the role of many possible genetic variants has been suggested, study into and validation of their pathogenicity is limited since conventional cellular models utilized in research labs do not properly recapitulate human physiology. A revolutionary discovery in 2007 allowed the generation of human induced pluripotent stem cells (hiPSCs) by reprogramming somatic cells from accessible human tissue to a pluripotent state. Soon thereafter, differentiation of these hiPSCs into cardiomyocytes (hiPSC-CMs) was accomplished and recognized and validated as a more physiological cellular model capable of mimicking fundamental phenotypic features of cardiac disease-associated variants. Recent advances in genetic engineering also enabled the genotype-phenotype correlation of a specific genetic variant to be investigated by comparing genetically-matched hiPSC lines. Clinical manifestation of congenital long QT syndrome type 2 (LQT2) can range from the absence of symptoms to life-threatening arrhythmia episodes. Insight into the genetic etiology could advance clinical-decision making and guide the development of tailored medicine strategies. The focus of this thesis was twofold, 1) to validate technical procedures to store hiPSC-CMs and 2) to investigate genetic variant pathogenicity and unravel variable disease expressivity in genetically-matched hiPSC-CM models with LQT2-associated variants.

Chapter 1 provides a general introduction to the thesis. The electrophysiology of each cardiomyocyte is tightly orchestrated by multiple cardiac ion channels and proteins in an adult healthy human heart. Changes in this electrophysiology balance are linked to specific congenital arrhythmic diseases and their corresponding mutations in cardiac ion channels or proteins. hiPSC-CMs are increasingly used to model phenotypic manifestation of genetic variants, including genetic modifiers and variants of unknown significance, and could provide insight into the variability in disease expressivity and mechanism of action. Finally, tailored methods allowing the detection of the key electrophysiological and contractile cell properties are addressed.

Chapter 2 reviews the past, present, and future of how hiPSCs could contribute to gaining insight into the mechanisms underlying monogenic cardiac diseases such as primary arrhythmias and cardiomyopathies to guide the field towards the development of more personalized therapies for individual patients.

In **Chapter 3**, we established cryopreservation as an opportune method to store hiPSC-CMs with molecular and functional characteristics being retained after thaw and recovery. Freezing of large-scale identical hiPSC-CM batches facilitates addressing various biomedical research challenges including model reproducibility, interlaboratory sharing of cells, and large-scale screening of pharmacological compounds.

One determinator for the differences in arrhythmic risk in LQT2 patients is the location of the mutation in *KCNH2*, which encodes the ion channel hERG and conducts the rapid delayed rectifier potassium current (I_{Kr}) in cardiomyocytes. In **Chapter 4**, we demonstrate the potential of hiPSC-CMs to reveal the inherent severity of *KCNH2* mutations when using genetically-matched lines with *KCNH2* mutations in the pore region exhibiting a more prolonged repolarization and increased susceptibility to proarrhythmic effects compared with the hiPSC-CMs with the tail mutation or unedited control.

Another aspect of the clinical heterogeneity in LQT2 is the potential contribution of additional genetic variants such as common single nucleotides (SNPs) to the disease phenotype, also defined as genetic-modifying variants. In **Chapter 5** we corroborate the capability of hiPSC-CMs to detect the impact of genetic modifiers. We demonstrate that the chromosomal phase of the KCNH2-K897T SNP does influence the biophysical properties of I_{Kr}, prolonging the repolarization phase, and increasing the drug-induced occurrence of arrhythmic events.

In **Chapter 6**, we provided insight into the electrophysiological impact of a compound heterozygous *KCNH2* mutation genotype found in some LQT2 patients with severe disease phenotypes. The hiPSC-CMs harboring a missense *KCNH2* mutation in one allele and a frameshift mutation in the other allele (compound heterozygosity), exhibited a complete loss of hERG compared to their matching isogenic heterozygous mutated hiPSC-CMs and resulted in a further depletion in I_{Kr} density, depolarized diastolic membrane potential, and altered AP shape. This more severe electrical phenotype suggest that there are potentially differences in pathogenicity and mechanism of action between distinct gene-dosage *KCNH2* mutations. Addressing this in further studies will improve the understanding of clinical manifestations of mutation-specific phenotypes.

Finally, **Chapter 7** we deliberate the results outlined in this thesis and provide future propositions to further advance the field of hiPSC-CM disease modeling in order to aid in bridging the gap between the current incomplete risk stratification and precision medicine approaches.