A humanized hypertrophic cardiomyopathy model to elucidate molecular mechanism in disease pathology

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Introduction

Hypertrophic cardiomyopathy (HCM) is estimated to be the most prevalent hereditary heart disease in the world. In HCM patients, the left ventricular wall of the heart thickens due to enlarged cardiomyocytes (heart cells). The disease is a major cause of disability and sudden cardiac death (SCD) in patients, particularly those induced by arrhythmia. In Singapore, it is the most prevalent genetic heart disease, affecting every 1 in 500 people. The mechanism of HCM remains poorly defined, requiring further understanding for improved therapeutic strategies. Due to the challenge of obtaining cardiac biopsies from human subjects, using induced pluripotent stem cells (iPSCs) technology, we successfully generated a humanized HCM model representative of an actual diseased heart cell to investigate the molecular mechanisms involved in the disease pathology and the link between arrhythmia and HCM.

Hypothesis and Methodology

Due to arrhythmia induced SCD being a profound manifestation of HCM, we hypothesize that ion channel irregularities are responsible for the symptoms and phenotype of HCM.

Results and Discussion

Characterization of iPSCs

iPSCs were generated from blood of a HCM patient. Their pluripotency was confirmed by conducting immunofluorescence staining of the following indicative markers: Oct4, a nuclear marker, and Tra-1-60; a surface marker.

Differentiation into cardiomyocytes

Control- and HCM-iPSCs were subjected to cardiac differentiation. Initially, iPSC colonies were dissociated into single cells and seeded into micro-wells. 24 hours later, these single cells had formed aggregates known as embryoid bodies (EBs). The EBs were taken out of the micro-wells and treated with specific growth factor and small molecules for 8 days after which they were plated. Contracting clusters were visible by day 14 of differentiation. Throughout cardiac differentiation, the EBs increased in size, particularly between days 2-4 and days 8-14.

Phenotypic characterization of HCM model

The sarcomere is the basic contractile unit within cardiomyocytes. Staining against α-actinin revealed disorganized sarcomeres in the HCM-iCMs as compared to Control-iCMs. Staining of the nucleus with DAPI revealed an enlarged nucleus in HCM-iCMs indicating that HCM-iCMs were probably undergoing cell division, due to re-initiation of the foetal program. Similar to the clinical manifestation, HCM-iCMs were larger in size as compared to Control-iCMs.

HCM-iCMs display calcium irregularities

Quantitative gene expression analysis indicated that levels of potassium channels (KCNQ1, KCNE1, KCNJ2) remained relatively similar between Control and HCM-iCMs, however, levels of calcium channels (RYR2, ATP2A2, CACNA1D, CACNA1C) were significantly up-regulated in HCM-iCMs. The levels of sarcomere genes (TNNT2 and MYL2) were also up-regulated in HCM-iCMs. Calcium ions regulate the contraction and relaxation of the cardiomyocytes. Calcium imaging suggested that there were significantly more irregular calcium transients among the HCM-iCMs as compared to Control-iCMs. These results indicate that HCM-iCMs have abnormal calcium handling properties which may give rise to arrhythmias in HCM patients.

Conclusions and Future Work

In conclusion, our results show that iPSC-iCMs are indeed a good human model for recapitulating the clinical manifestations of HCM, allowing us to observe the disease at a molecular level to further understand its mechanisms. Our results have proven through examination of the relevant genes and calcium imaging that HCM-iCMs are found to have abnormal calcium handling properties, leading to arrhythmia. Using our model, future work can be done to develop treatments that can stop or resolve the calcium irregularities, and hence effectively lead to a decrease in the disease phenotypes without obtaining actual cardiomyocytes. Our research has laid the foundation and direction for potential treatments and research to be done, pointing towards multiple extensions of our work that will increase our knowledge and efficiency at treating HCM and potentially other diseases as well.

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