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.

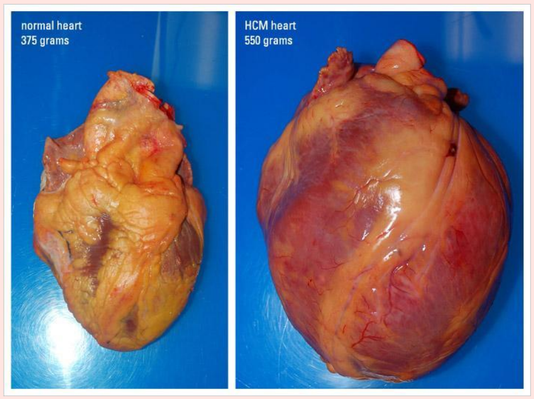
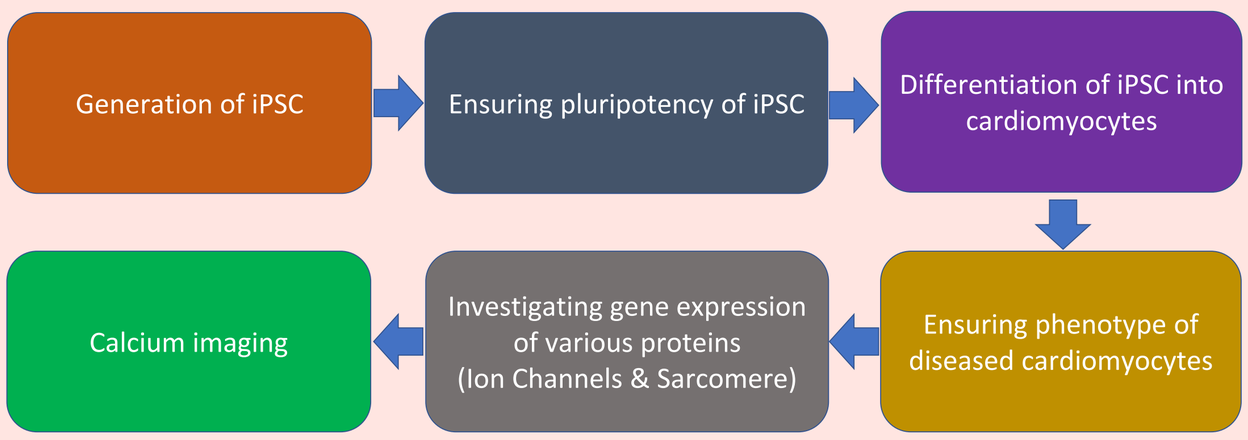


Figure 1. Image showing the contrast between a healthy patient's heart (left) and a heart of a patient suffering from HCM (right).

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.

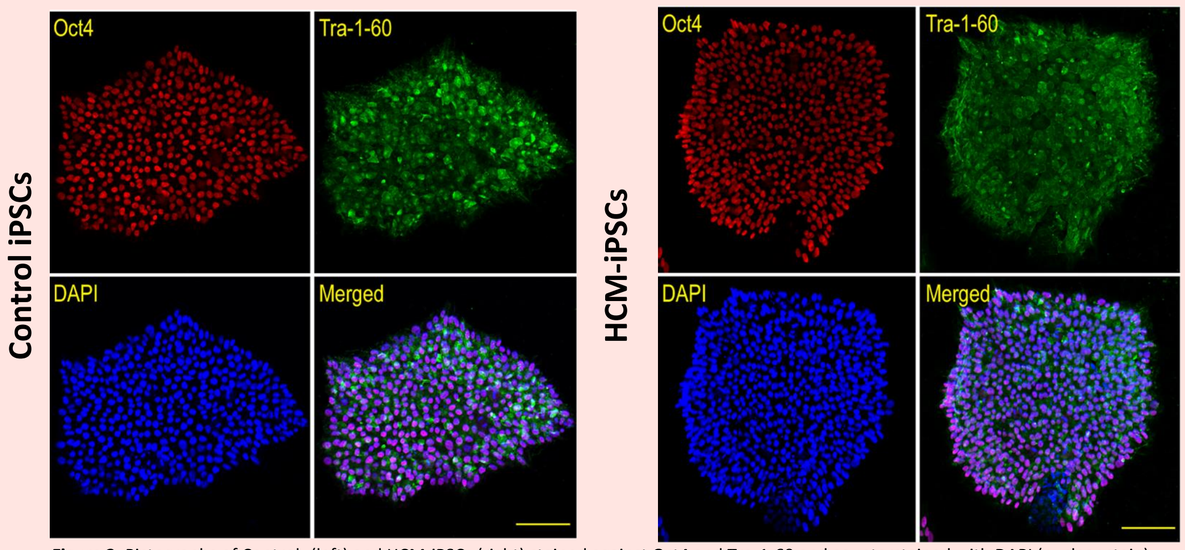


Figure 2. Pictographs of Control- (left) and HCM-iPSCs (right) stained against Oct4 and Tra-1-60 and counterstained with DAPI (nuclear stain). Note the nuclear localization of Oct4 and the surface expression of Tra-1-60. Scale bar: 100 µm.

Immunostaining revealed that both Control- and HCM-iPSCs were **pluripotent** as they **stained positive** for these markers

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.**

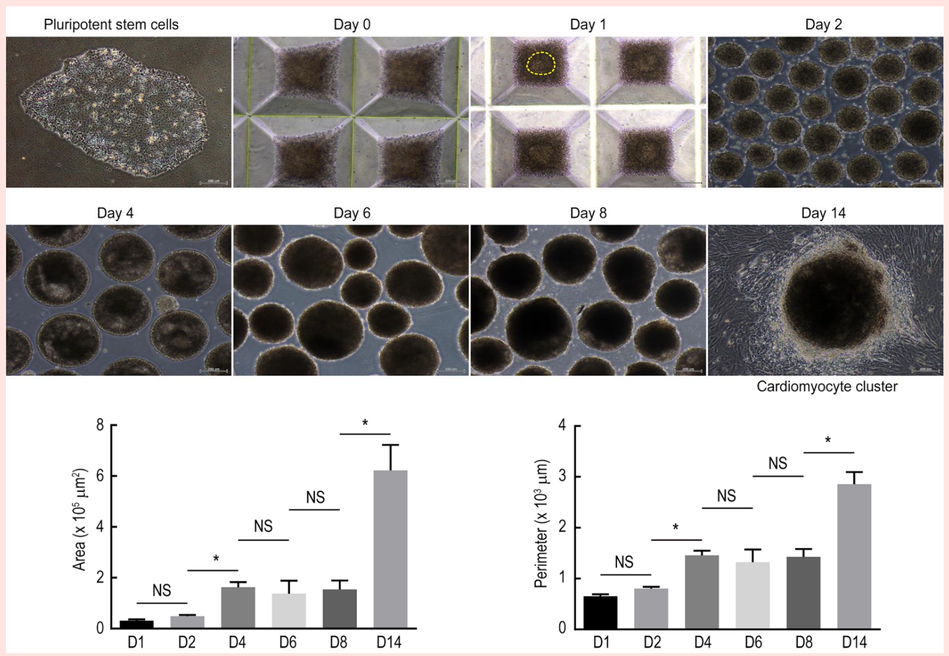


Figure 3. Top panel; Pictographs of the cardiac differentiation procedure from day 0 to day 14. Note that not every single iPSC is incorporated into the EB. The yellow dotted circle highlights an aggregated EB. Bottom panel; Bar graphs showing changes in area and perimeter of the EBs (n=5) throughout cardiac differentiation. Bar graphs presented as mean ± SD. *p<0.05 significantly different between comparison groups. Abbreviation: NS- non-significant, D- day

Phenotypic characterization of HCM model

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

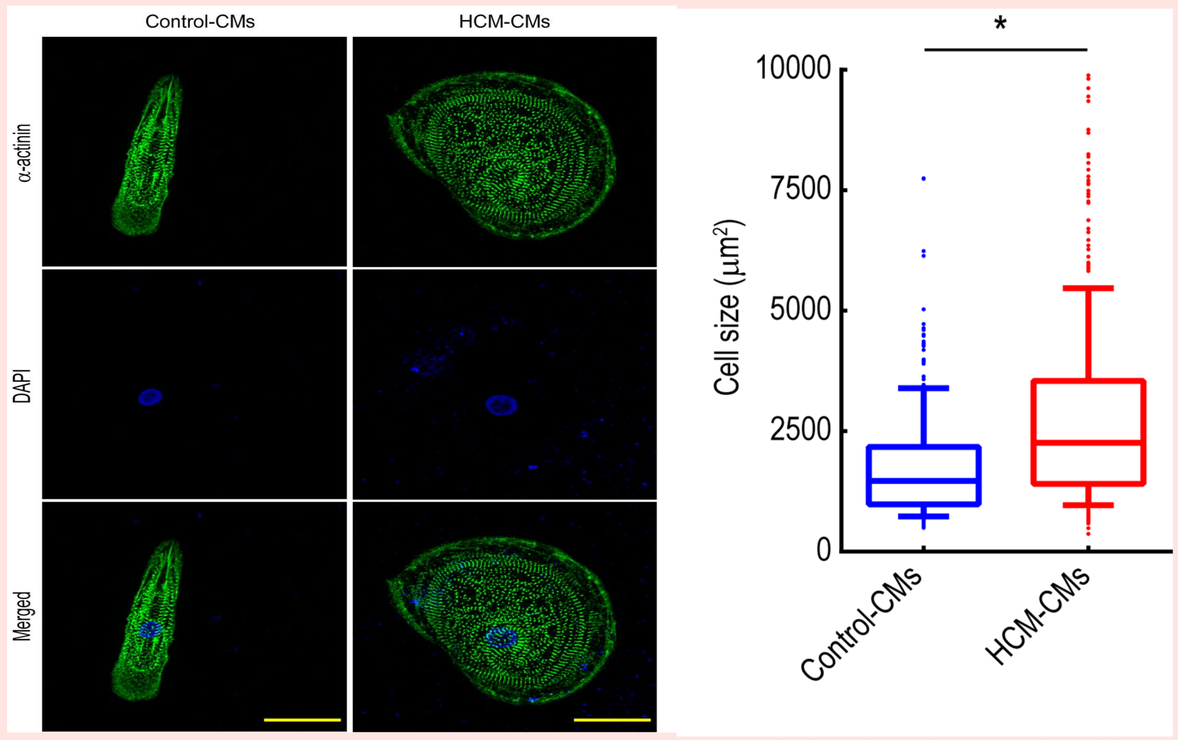


Figure 4. Phenotypic analysis of Control- and HCM-CMs. Left panel; pictographs of Control- and HCM-CMs stained against sarcomeric α-actinin and counterstained with DAPI. Note the disorganized sarcomeres and enlarged nucleus in HCM-CMs. Scale bar: 50 µm. Right panel; Box and Whisker plots showing differences in cell size between Control- and HCM-CMs. Box and Whisker plots presented as median with 10-90 percentile range. (n=100). *p<0.05 significantly different in comparison to Control-CMs.

HCM-CMs display calcium irregularities

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

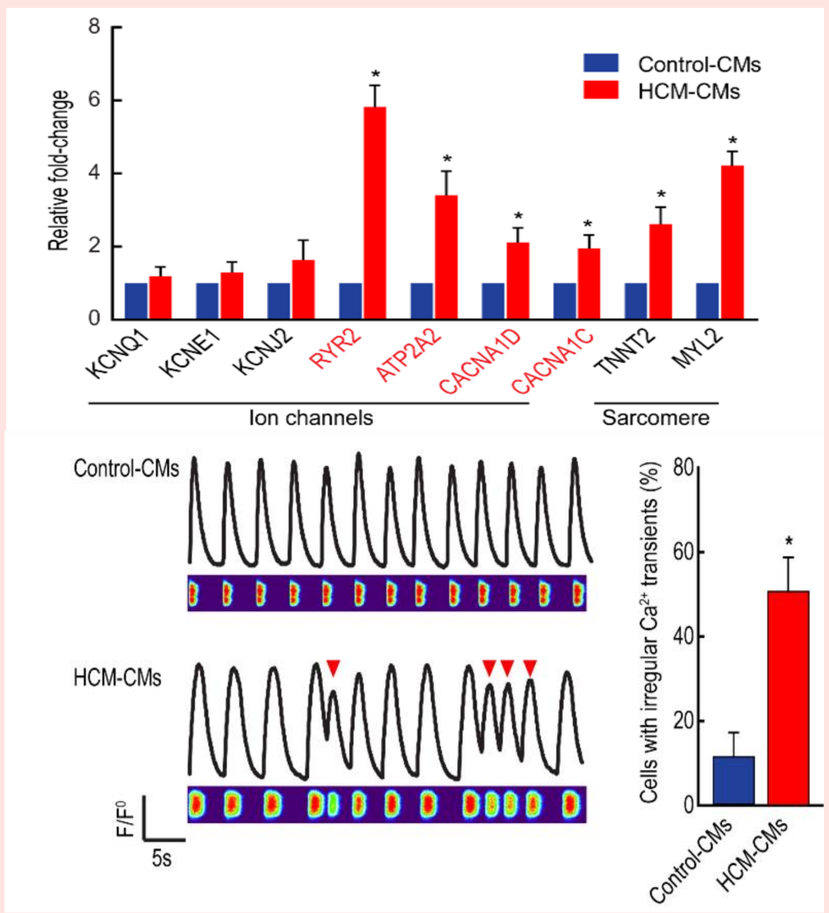


Figure 5. Gene expression analysis and calcium imaging of Control- and HCM-CMs. Top panel; Bar graphs showing differences in expression profile of 9 various genes in Control- and HCM-CMs. Bar graphs presented as mean ± SD (n=3 independent experiments). Calcium channel are annotated in red font. Bottom panel; Left; representative images of spontaneous calcium traces in Control- and HCM-CMs. Note the irregular calcium transients (red arrowheads) in HCM-CMs. Right; bar graph showing differences in prevalence of calcium irregularities between Control- and HCM-CMs. Bar graph presented as mean ± SD (n=50 cells analysed per group). *p<0.05 significantly different in comparison to Control-CMs.

Conclusions and Future Work

In conclusion, our results show that iPSC-CMs 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-CMs 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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