LETTERS TO THE EDITOR

doi:10.1093/cvr/cvv038
Published online 17 February 2015

Prolonged action potentials in HCM-derived iPSC – biology or artefact?

With great interest we read the article by Han et al. ‘Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells’ recently published in ‘Cardiovascular Research’.1 Life-threatening ventricular arrhythmias in hypertrophic cardiomyopathy (HCM) remain a challenge not only for clinical management of individual patients, but also from the viewpoint of cellular cardiac electrophysiology. It remains an enigma how mutations of sarcomeric proteins can increase the propensity for arrhythmias. A recent concept suggests that the often increased Ca$$^{2+}$$-binding affinity of mutated sarcomeres in HCM increases Ca$$^{2+}$$ buffering and, by diastolic release of Ca$$^{2+}$$, the propensity for arrhythmias.2 The advantage of patient-specific induced pluripotent stem cells (iPSC) should be that effects of patient-relevant mutations can be studied in the correct genetic and cellular background. With the rich toolbox of experimental electrophysiology at hand, iPSC-derived cardiac myocytes can be evaluated without restriction in time and numbers. The critical and unanswered question is whether (im)maturing iPSC-derived cardiac myocytes really reflect the phenotype of the patient or predict it. Data answering this question are lacking also in the recent study.

Han et al. report six-fold higher sodium current density and almost two times higher APD$$\text{90}$$ in HCM-derived iPSC cardiac myocytes compared with those from healthy controls. Both numbers appear somewhat surprising. There are few data recording Na$$^{+}$$ currents in human ventricular myocytes. Data indicate that different disease processes (congenital heart disease vs. cardiomyopathies, terminal heart failure vs. non-failing) do not affect sodium currents.3 However, it should be noted that this work not specifically addressed HCM. Nevertheless, calculations with a well-established computer model for human ventricular AP$$^{+}$$ predict a six-fold higher peak sodium current to cause three-fold higher maximum upstroke velocity, but unchanged APD$$\text{90}$$ (own unpublished data). Unfortunately, data on upstroke velocity are lacking in the current paper by Han et al.1 Transient outward potassium currents depicted in Figure S1 likely indicate peak currents of I$$\text{to}$$ (no methodological details are given) and show a 25% increase in HCM-derived iPSC cardiac myocytes. Recent work in dog ventricular myocardium suggests that 90% block of I$$\text{to}$$ can evoke marked APD prolongation when other potassium channels are blocked.3 Therefore, we would rather expect shortened rather than prolonged APD because of increased I$$\text{to}$$. However, the computer model mentioned above suggest that such small increase in I$$\text{to}$$ should not affect APD$$\text{90}$$. Only the slight increase in t-type Ca$$^{2+}$$-current density by ~50% would substantially prolong APD$$\text{90}$$ (+9% in our computer simulation). Thus, the measurements of ion currents and APD$$\text{90}$$ do not correspond to each other when applying an established computer model, indicating

(1) either that repolarization in HCM-derived iPSC cardiac myocytes has little resemblance with that of mature undesised ventricular myocytes (as reflected by experimental obtained from dog or computer models), or
(2) that this particular patient shows more, not yet identified abnormalities in the delicate balance between depolarizing and repolarizing forces.

Increases in APD$$\text{90}$$ at a magnitude described herein are expected to correlate with markedly prolonged QT-interval in the ECG of the respective patient. No such information is provided in the paper by Han et al. With regard to clinical implications of this study, it is important to note that marked QT prolongation is not a regular phenotype in patients with HCM. In the largest study into this question, QT$$^{+}$$ was only mildly associated with the extent of hypertrophy.4 In summary, the available clinical data argue against an LQT mechanism of HCM-related arrhythmias, and we believe that it is premature to conclude from the iPSC data obtained from just one patient that the conduction delay and repolarization abnormalities in HCM result from changes of ionic currents and action potential duration.

Conflict of interest: none declared.

References

1. Han L, Li Y, Tchao J, Kaplan AD, Lin B, Li Y, Mich-Basso J, Lis A, Hassan N, London B, Tobita K, Rasmusson RL, Yang L. Study familial hypertrophic cardiomyopathy in induced pluripotent stem cells.1 Questions were raised about our experimental system, based on unpublished findings using simulations from a computer model of human ventricular myocytes. Christ et al. conclude that discrepancies between the iPSC-derived cardiomyocytes and the computer model indicate either a peculiarity in the specific human we studied or a problem with iPSC-derived cardiomyocytes in general. We

doi:10.1093/cvr/cvv039
Published online 17 February 2015

Reconciling computer models and stem cell models of human cardiac repolarization: reply

We appreciate the interest in our recent study of the familial hypertrophic cardiomyopathy in induced pluripotent stem cells.1 Questions were raised about our experimental system, based on unpublished findings using simulations from a computer model of human ventricular myocytes. Christ et al. conclude that discrepancies between the iPSC-derived cardiomyocytes and the computer model indicate either a peculiarity in the specific human we studied or a problem with iPSC-derived cardiomyocytes in general. We
ready agree that some properties in our HCM cells may be patient specific: this is part of the promise of personalized medicine. However, we propose an alternative interpretation for the discrepancy between iPSC-derived cardiomyocytes and model simulations: the model is not an accurate representation of the cardiomyocytes under study. Nevertheless, computer models can, and should, be used in conjunction with these cells to further our understanding of human genetic disorders.

Christ et al. commented on the relationship between action potential upstroke and \( \Delta V_{\text{max}} \) magnitude. Some data on action potential upstroke (dV/ dt\(_{\text{max}}\)) were included in the supplementary data. However, dV/ dt\(_{\text{max}}\) for both control and HCM cardiomyocytes were much slower than one would expect for freshly isolated cardiomyocytes. This is a consequence of the depolarized resting potential of iPSC-derived cardiomyocytes, which inactivates \( h_{\text{Na}} \), and thus reduces upstroke velocity. The depolarized membrane potential in iPSC-derived cardiomyocytes is due to lack of \( I_{\text{K1}} \). We used a Cybercyte (Cytocytometrics) to electronically express a mathematical representation of \( I_{\text{K1}} \) in the iPSC,\(^2\) thus combining experimental and mathematical modelling (Table 1). Expression of \( I_{\text{K1}} \) returns the resting potential to physiological levels and reduces inactivation of \( h_{\text{Na}} \). Table 1 shows that there is a statistically significant increase in AP upstroke associated with the larger sodium current in HCM under these conditions.

Modelling is also critically important when describing discrepancies between what we think we know about a system and the way it actually behaves. The inability to reproduce changes in repolarization through modulation of simulated current densities, perhaps reflects a limitation of the models. Additional factors, such as the slow or late component of sodium channel inactivation, may need more detailed biophysical characterization in the model. In fact, the computer modelling paper cited has three separate formulations of \( h_{\text{Na}} \), with no definitive representation. Clearly, closer examination of current kinetic changes in iPSC-derived and native cardiomyocytes is needed, particularly of late sodium currents, as this has been implicated in human HCM acutely isolated from ventricular muscle.\(^3\) However, Chris et al. were stimulating cells with \( I_{\text{K1}} \) in the computer modelling study, whereas our paper analysed control and HCM spontaneous activity for AP prolongation. When we electronically expressed \( I_{\text{K1}} \) in these cells, the significant differences in AP duration between HCM and control cells were lost. The question for modelling becomes: is this difference due to changes in sodium and calcium currents, consequent to sodium loading and increased inward currents (e.g. sodium calcium exchange)? Or do the minor changes in other currents, such as \( I_{\text{Ca}} \) and \( I_{\text{L}} \), become amplified in the presence of reduced repolarization reserve? Interestingly, the recent study of Coppini et al.\(^3\) suggests such arrhythmogenic synergies in their acutely isolated human ventricular myocytes which have reduced \( I_{\text{K1}} \).

iPSC-derived cardiomyocytes not only offer a unique pathway to understanding patient-specific phenotypes, but also give us a strong test of our understanding of cardiac repolarization. Despite their potential limitations, iPSC-derived cardiomyocytes provide abundant, disease-related, reproducible, well-defined human cells. Fundamentally, we can measure what we currently understand as all the most critical parameters for repolarization in these cells. When simulations and data conflict, they highlight a discordance between what we currently understand and cellular reality. Used iteratively, computer modelling and stem cells, combined with the limited data available on native human myocytes, is a very powerful approach for advancing our understanding of human cardiac repolarization and disease.

**Table 1** Action potential parameters for control and HCM myocytes change with electronic expression of \( I_{\text{K1}} \)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 22)</th>
<th>(+I_{\text{K1}})</th>
<th>HCM (n = 45)</th>
<th>(+I_{\text{K1}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>−56.00 ± 2.69</td>
<td>−83.54 ± 0.66</td>
<td>−58.16 ± 1.78</td>
<td>((P = 0.498))</td>
</tr>
<tr>
<td>dV/dt(_{\text{max}}) (mV/ms)</td>
<td>79.00 ± 2.80</td>
<td>136.00 ± 5.78</td>
<td>86.70 ± 4.34</td>
<td>((P = 0.242))</td>
</tr>
<tr>
<td>APD90 (ms)</td>
<td>560.50 ± 81.32</td>
<td>366.73 ± 66.87</td>
<td>910.38 ± 86.86</td>
<td>((P = 0.007))</td>
</tr>
<tr>
<td>APD50 (ms)</td>
<td>429.33 ± 84.10</td>
<td>323.61 ± 66.77</td>
<td>756.36 ± 82.22</td>
<td>((P = 0.00882))</td>
</tr>
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This table demonstrates the effects that expression of a constant amount of \( I_{\text{K1}} \) had on cellular phenotypes for control and HCM cells. After expression of \( I_{\text{K1}} \), there is a significant increase in dV/dt\(_{\text{max}}\), and the significant differences in action potential duration are eliminated. Cardiac action potentials were recorded from single myocytes using standard whole-cell patch-clamp techniques at room temperature using Axopatch1D, Digidata 1322A, and pClamp 9 (Axon Instruments) for data amplification, acquisition, and analysis. Cells in Tyrode solution were kept in a recording (pL volume) chamber and were (300) continuously perfused with fresh Tyrode solution. Suction pipettes were fabricated from borosilicate glass using a Flaming/ Brown horizontal. \( \Omega \)-Aps were micropipette pull with resistances between 2 and 4 \( \Omega \) elicited by a current injection through the patch, sufficient to elicit a peak. Patch pipettes contained the following intracellular solution (mM): 140 KCl, 5 EGTA, 5 ATP (Mg salt), 5 Na\(_2\)-creatinephosphate, 0.2 GTP, and 10 HEPES, pH 7.4 and extracellular solution contained (mM): 144 NaCl, 5.4 KCl, 1 MgCl\(_2\), 2.5 CaCl\(_2\), 5.6 glucose, and 10 HEPES, pH 7.4. Data were acquired at 20 kHz and filtered at 2 kHz using Axopatch1D, Digidata 1322A, and pClamp 9. \( I_{\text{K1}} \) was digitally expressed in the myocytes using Cybercyte (Cytocytometrics) as previously reported.\(^1\) Action potential parameters are reported as mean ± standard error of the mean. In parentheses, the differences were analysed using a t-test.

**References**


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