IPSC-derived cardiomyocytes as a model for studying mechanisms of repolarization and disease in the developing heart: focus on hERG isoforms

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Perturbation of cardiac I_{Kr} has been well established as the basis of acquired long QT syndrome (aLQTS) and an inherited form of the disease, LQT2 (Curran *et al.*, 1995; Sanguinetti *et al.*, 1995; Trudeau *et al.*, 1995). Mutations in the encoding gene, *hERG* or *KCNH2*, often strike the young and represent a major cause of sudden cardiac death. Increasingly, LQTS is considered an important underlying cause of sudden infant death syndrome (SIDS) (Rhodes *et al.*, 2008; Crotti *et al.*, 2013). Such developments warrant major efforts to understand the determinants of cardiac arrhythmia in these youngest of patients.

Previous studies have revealed that the channels producing cardiac I_{Kr} comprise two subunits, hERG 1a and 1b (Jones *et al.*, 2004; London *et al.*, 1997; Sale *et al.*, 2008). In heterologous expression systems, heteromeric 1a/1b channels produce twice as much current during the action potential as 1a homomers, owing to altered gating kinetics conferred by the 1b subunit (Sale *et al.*, 2008). Thus, loss of 1b is expected to dramatically reduce I_{Kr} *in vivo*. Consistent with this prediction, a hERG 1b-specific mutation identified in an 8-year-old LQTS patient caused a loss of 1b expression (Sale *et al.*, 2008). A similar phenotype was described in a more recent study that attributed a case of intrauterine fetal death to a unique 1b mutation (Crotti *et al.*, 2013).

Our current efforts are focused on evaluating the role of hERG isoforms in the developing human heart using, as a model, cardiomyocytes (CM's) derived from induced pluripotent stem cells (iPCS's). Work from several labs suggests that these cells share characteristics of cardiomyocytes from the embryonic, fetal or neonatal stages (Lieu *et al.*, 2013; Ma *et al.*, 2011; Doss *et al.*, 2012). Compared to adult cardiomyocytes, the iPSC-derived CM's have a depolarized mean diastolic potential due at least in part to reduced levels of expression of I_{K1}. We have recorded from iPSC-derived CM's (Cellular Dynamics International, Madison, WI) in small beating clusters at approximately 21-32 days after plating using perforated patch current clamp at 37°C. Most cells (20/29) have characteristics of ventricular myocytes with APD_{90} = 462 ± 35 ms and plateaus represented by the ratio $(APD_{30}\text{-}APD_{40})/(APD_{70}\text{-}APD_{80})$ = 2.7 ± 0.3. As reported by others (Doss *et al.*, 2012),we found a depolarized mean diastolic potential of -63.9 ± 2.7 mV, likely reflecting low levels of I_{K1}. E-4031, a specific I_{Kr} blocker, caused a further and dramatic depolarization of the mean diastolic potential, suggesting that in the absence of I_{K1}, I_{Kr} is the predominant repolarizing force. E-4031 also prolonged action potential duration (APD) and triggered early afterdepolarizations (EAD's). At higher concentrations, E-4031 triggered oscillations reminiscent of torsades de pointes arrhythmia, in some cases leading to a depolarizing block of activity.

To determine the contribution of hERG isoforms to CM function, we used qRT-PCR and found robust expression of alternate transcripts encoding both the hERG 1a and 1b isoforms. We transfected cardiomyocytes with a 1b-specific shRNA, which reduced hERG 1b transcript to about 60% of its original value (in both HEK-293 cells and CM's). We found that the mean diastolic potential was significantly depolarized in the transduced cells compared with mock-transfected controls (−59.7 ± 4 mV, n = 17; −71.6 ± 2.9 mV, n = 8, respectively; p = 0.025), similar to one of the effects of E-4031 and consistent with a reduction in I_{Kr}. However, rather than lengthening APD, 1b knockdown shortened APD, reduced the plateau, and increased firing frequency. Interestingly, intrauterine LQTS is associated with bouts of tachycardia (Crotti *et al.*, 2013; Cuneo *et al.*, 2008) which may correspond to the increased firing rates observed with 1b knockdown. Ongoing efforts are aimed at determining the differences between the knockdown phenotype and the E-4031-induced behavior. For example, the effect of knockdown in the cell patched by the electrode may be dampened by the normal behavior of untransfected CM's physically and electrotonically attached. Alternatively, knockdown of the 1b isoform may lead to electrical remodeling to which the E-4031-treated CM is not subjected.

Crotti L, Tester DJ, White WM, Bartos DC, Insolia R, Besana A, Kunic JD, Will ML, Velasco EJ, Bair JJ, Ghidoni A, Cetin I, Van Dyke DL, Wick MJ, Brost B, Delisle BP, Facchinetti F, George AL, Schwartz PJ & Ackerman MJ. (2013) *Journal of the American Medical Association* **309**, 1473-82.

Cuneo BF, Strasburger JF & Wakai RT. (2008) *Journal of Electrocardiology* **41**, 116.e111-16, doi: 10.1016/j.jelectrocard.2007.12.010

Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED & Keating MT. (1995) *Cell* **80**, 795-803.

Doss MX, Di Diego JM, Goodrow RJ, Wu Y, Cordeiro JM, Nesterenko VV, Barajas-Martinez H, Hu D, Urrutia J, Desai M, Treat JA, Sachinidis A & Antzelevitch C. (2012) *PloS one* **7**, e40288, doi: 10.1371/journal.pone.0040288

Jones EM, Roti Roti EC, Wang J, Delfosse SA & Robertson GA. (2004) *Jounal of Biological Chemistry* **279**, 44690-94.

Lieu DK, Fu JD, Chiamvimonvat N, Tung KC, McNerney GP, Huser T, Keller G, Kong CW & Li RA.(2013) *Circulation: Arrhythmia and Electrophysiology* **6:** 191-201.

London B, Trudeau MC, Newton KP, Beyer AK, Copeland NG, Gilbert DJ, Jenkins NA, Satler CA & Robertson GA. (1997) *Circulation Research*. **81**, 870-78

Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, Kolaja KL, Swanson BJ & January CT. (2011) *American Journal of Physiology: Heart and Circulatory Physiology*. **301**, H2006-17

Rhodes TE, Abraham RL, Welch RC, Vanoye CG, Crotti L, Arnestad M, Insolia R, Pedrazzini M, Ferrandi C, Vege A, Rognum T, Roden DM, Schwartz PJ & George AL. (2008) *Journal of Molecular and Cellular Cardiology* **44**, 571-81.

Sale H, Wang J, O'Hara TJ, Tester DJ, Phartiyal P, He JQ, Rudy Y, Ackerman MJ & Robertson GA. (2008) *Circulation Research* **103**, e81-95.

Sanguinetti MC, Jiang C, Curran ME & Keating MT. (1995) *Cell* **81**, 299-307.

Trudeau MC, Warmke JW, Ganetzky B & Robertson GA. (1995) *Science* **269**, 92-95.

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