hERG 1b is critical for human cardiac repolarization

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The human ether-à-go-go-related gene (*hERG*; or *KCNH2*) encodes the voltage-gated potassium channel underlying I_{Kr} , a repolarizing current in the heart. Mutations in *KCNH2* or pharmacological agents that reduce I_{Kr} slow action potential (AP) repolarization and can trigger cardiac arrhythmias associated with long QT syndrome. Two channel-forming subunits encoded by *KCNH2* (*hERG* 1a and 1b) are expressed in cardiac tissue. In heterologous expression systems, these subunits avidly coassemble and exhibit biophysical and pharmacological properties distinct from those of homomeric *hERG* 1a channels. Despite these findings, adoption of *hERG* 1a/1b heteromeric channels as a model for cardiac I_{Kr} has been hampered by the lack of evidence for a direct functional role for the 1b subunit in native tissue. In this study, we measured I_{Kr} and APs at physiological temperature in cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs). We found that specific knockdown of the 1b subunit using shRNA caused reductions in 1b mRNA, 1b protein levels, and I_{Kr} magnitude by roughly one-half. AP duration was increased and AP variability was enhanced relative to controls. Early afterdepolarizations, considered cellular substrates for arrhythmia, were also observed in cells with reduced 1b expression. Similar behavior was elicited when channels were effectively converted from heteromers to 1a homomers by expressing a fragment corresponding to the 1a-specific N-terminal Per-Arnt-Sim domain, which is omitted from *hERG* 1b by alternate transcription. These findings establish that *hERG* 1b is critical for normal repolarization and that loss of 1b is proarrhythmic in human cardiac cells.

hERG | *KCNH2* | arrhythmia | early afterdepolarization | long QT syndrome

The human ether-à-go-go-related gene (*hERG*; or *KCNH2*) encodes the voltage-gated potassium channel underlying a native cardiac current known as I_{Kr} (1, 2). Mutations in the gene or drugs that block the channel can diminish I_{Kr} and slow ventricular repolarization, thus prolonging the QT interval on the surface electrocardiogram and causing long QT syndrome (LQTS) (1–3). Individuals with LQTS have an increased risk for torsades de pointes arrhythmia, syncope, and sudden cardiac death. Since 2005, a cell-based assay expressing the *hERG* 1a isoform as a proxy for I_{Kr} has been the cornerstone of drug safety screening for new drug development (4). Thus, whether current drug safety assays accurately represent the native channel is of paramount importance.

The first studies of heterologously expressed *hERG* channels described biophysical (1, 2) and pharmacological (2, 5) properties uniquely characteristic of cardiac I_{Kr} . Subsequently, alternate transcripts of *KCNH2* in mouse and human heart were shown to encode two subunits: 1a (the original isolate) and 1b (6, 7). In the *hERG* 1b transcript, an alternate 5' exon replaces 1a exons 1–5, resulting in a shorter, unique N terminus that lacks a Per-Arnt-Sim (PAS) domain (also known as the ether-à-go-go domain) (8, 9). In heterologous systems, *hERG* 1b subunits avidly associate with *hERG* 1a but fail as homomers to traffic efficiently to the plasma membrane (10). Compared with homomeric 1a currents, *hERG* 1a/1b currents exhibit a twofold increase in the rates of activation, recovery from inactivation, and deactivation. During a voltage-clamp command mimicking a cardiac action potential (AP), these gating differences result in a nearly twofold increase in the repolarizing current integral (11).

Surprisingly, the changes in gating also lead to differences in drug sensitivities (IC_{50} values) by as much as eightfold (12). Overall, the characteristics of 1a/1b current properties appear to better resemble those of native I_{Kr} (11, 13, 14).

Heteromeric 1a/1b currents can be converted to 1a-like currents by coexpressing a fragment representing the PAS domain (15). The PAS domain interacts directly with the heteromeric channel, presumably occupying an empty PAS domain receptor site left available by the abbreviated *hERG* 1b N terminus (15, 16). The resulting currents are smaller in amplitude owing to decreased rates of activation and recovery from inactivation characteristic of 1a currents, and they deactivate more slowly (15). Homomeric 1a currents are unaffected by the PAS fragments, as expected if all PAS receptor sites are occupied. Thus, these differences in gating kinetics arise because the 1b N terminus lacks the PAS domain and not because of an effect conferred by the unique N-terminal sequence of 1b (15).

Both 1a and 1b subunits are expressed in human ventricle (17), but to date, genetic evidence for 1b-specific disease mutations remains limited to two clinical cases (11, 18). Thus, direct evidence for a functional role of *hERG* 1b in human cardiomyocytes is lacking. In this study, two independent approaches were used to alter the contribution of *hERG* 1b: transfection of shRNA to reduce 1b levels, and exogenous expression of the 1a-specific PAS domain fragment to modify extant 1a/1b channels. We found that reducing *hERG* 1b subunit levels, or effectively converting 1a/1b channels to 1a-like channels with the 1a PAS domain, altered I_{Kr} kinetics and gave rise to cellular manifestations of proarrhythmia in ventricular-like cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs). Thus, the *hERG* 1b subunit is

Significance

The 1a subunit of the human ether-à-go-go-related gene (*hERG*) potassium channel is a critical component of cardiac repolarization and the cornerstone of safety screens for new drug development. A second subunit, 1b, coassembles with 1a and modifies channel gating and drug block sensitivity. Adoption of 1a/1b heteromers as a model of native *hERG* current, I_{Kr} , has been hampered by the absence of direct evidence that 1b contributes to human cardiac repolarization. This study provides the first functional evidence, to our knowledge, that 1a/1b channels rather than homomeric 1a channels mediate repolarization. Because heteromeric and homomeric *hERG* channels have different pharmacological profiles, these findings have implications for native I_{Kr} models and *hERG*-based drug safety tests that help protect against drug-induced sudden cardiac death.

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essential to normal I_{Kr} kinetics and magnitude as well as cardiac rhythmicity.

Results

hERG 1b Is Expressed in iPSC-CMs. We first determined whether hERG 1b was expressed in iPSC-CMs. Immunocytochemistry with a 1b-specific antibody revealed robust fluorescence that was reduced by approximately one-half in the presence of 1b-specific shRNA (Fig. 1*A–D*). Mock-transfected iPSC-CMs served as the control. Signal intensity was quantified using linear fluorescence profiles for hERG 1b and actin, which were integrated and normalized to cell width. shRNA transfection significantly reduced hERG 1b fluorescence by 54.3% (Fig. 1*D*) but had no effect on actin fluorescence (Fig. S1*A*). Quantitative RT-PCR showed independently that the shRNA reduced hERG 1b mRNA in the cardiomyocytes by $46.6 \pm 1.6\%$ (Fig. 1*E* and Fig. S1, HEK cells). hERG 1a mRNA was also reduced but to a significantly smaller degree ($23.1 \pm 1.4\%$) (Fig. 1*E*). Transfection with a nontargeting shRNA control had no effect on mRNA levels of either hERG subunit (Fig. S1). These data show that hERG 1b is present in human iPSC-CMs at both the mRNA and protein levels, which was expected from studies of human ventricular myocardium (17, 18) and embryonic stem cell-derived cardiomyocytes (19). Furthermore, shRNA transfection predominantly targeted hERG 1b mRNA and reduced 1b mRNA and protein by roughly one-half.

hERG 1b Modulation Alters Native I_{Kr} . Next, we recorded membrane currents in iPSC-CMs using the whole-cell patch-clamp technique to assess the contribution of hERG 1b to native I_{Kr} . Fig. 2 shows that shRNA-mediated reduction in 1b caused a corresponding reduction of peak tail I_{Kr} density by 50%, from 1.4 ± 0.2 pA/pF in mock-transfected controls to 0.7 ± 0.2 pA/pF in shRNA-transfected cells (Fig. 2*A* and *B*) ($P = 0.02$). Deactivation, measured from the tail currents, was also slowed (control: 383 ± 66 ms, $n = 8$; shRNA: 887 ± 121 ms, $n = 7$; $P = 0.01$) (Fig. 2*C* and *D*), consistent with a reduced contribution of 1b subunits and the emergence of homomeric 1a-like current properties. The deactivation rates were intermediate between those of homomeric 1a and heteromeric 1a/1b channels (Fig. 2*D*), consistent with the incomplete reduction of hERG 1b

Fig. 1. shRNA reduces hERG 1b expression levels. iPSC-CMs immunostained for hERG 1b (magenta) and actin (green; *A*) under mock-transfected control conditions or (*B*) after transfection with 1b shRNA. (*C*) Sample linear fluorescence intensity profiles displaying hERG 1b fluorescence in control (blue) and shRNA-transfected (orange) iPSC-CMs. [Scale bar: 10 arbitrary units (AUs) $\times$ 5 μ m.] (*D*) Plot of average fluorescence intensity in control (336.3 ± 24.5 AU/ μ m; $n = 20$) and shRNA-transfected (153.7 ± 11.8 AU/ μ m; $n = 20$; $P < 0.001$) cells. (*E*) Plot of hERG 1a and 1b mRNA levels amplified with qRT-PCR in 1b shRNA-transfected cells normalized to those in untransfected cells ($n = 10$; $P < 0.0001$). Error bars are $\pm$ SEM. *Statistical significance relative to controls.

Fig. 2. hERG 1b modulation alters native I_{Kr} . (*A*) Sample E-4031-sensitive traces representing endogenous I_{Kr} from (Top) control, (Middle) shRNA-transfected, and (Bottom) PAS-infected iPSC-CMs. Pulses from -40 to 20 mV in 10 -mV steps are shown. The final 3 s of the 6 -s tail pulse are omitted for clarity. (Scale bar: 0.5 pA/pF $\times$ 1 s.) (*B*) Peak tail current density measured at -40 mV, plotted as a function of prepulse potential, and fitted with a Boltzmann function for control (black squares), shRNA-transfected (blue circles), and PAS-infected (green triangles). (*C*) Normalized samples of (Upper) -40 -mV tail current traces of native I_{Kr} , and (Lower) hERG currents overexpressed in iPSC-CMs. (Scale bar: 1 s.) (*D*) Weighted time constants of deactivation (τ_w) for native I_{Kr} (control, shRNA, and PAS) compared with overexpressed 1a and 1a/1b currents in iPSC-CMs. *Statistical significance at $P < 0.05$.

mRNA in these cells. The smaller but measurable reduction of 1a mRNA by the shRNA would also contribute to current reduction but not the observed kinetic changes.

To independently test the hypothesis that heteromeric hERG channels are responsible for native I_{Kr} , we exogenously expressed a fragment encoding the 1a-specific PAS domain in iPSC-CMs. Consistent with the corresponding experiment on hERG 1a/1b channels expressed in HEK293 cells, native I_{Kr} was transformed into a 1a homomeric-like current (15). Expression of the PAS fragment in iPSC-CMs significantly slowed deactivation compared with that in controls ($P < 0.0001$) and comparable with that in iPSC-CMs exogenously expressing hERG 1a (Fig. 2*D*). Neither peak tail current magnitude nor voltage dependence of native I_{Kr} was significantly affected (Fig. 2*B* and Table S1). Compared with the effects of 1b knockdown, PAS fragment expression more effectively converted I_{Kr} to 1a-like currents. Presumably, the reduced current levels resulting from RNAi reflect a reduction in channel surface density (Fig. 1), which was unaffected in cells expressing the PAS domain. Together, the results from shRNA knockdown and PAS expression unequivocally show that hERG 1b is expressed and contributes to I_{Kr} in human cardiomyocytes.

hERG 1b Modulation Impairs AP Repolarization. Previous studies showed that hERG 1a/1b channels are less rectifying owing to their faster activation and recovery from inactivation rates, and thus, compared with 1a homomers, they should contribute more current during repolarization (11). To test this hypothesis and assess the contributions of the hERG 1b subunit to AP morphology, we

recorded APs from iPSC-CM microclusters of 3–10 cells using perforated patch current clamp (K_{ATP} -transduced; see *Methods*, *SI Methods*, and *Fig. S2*). Like myocardium-derived cardiomyocytes, in the presence of E-4031 (an I_{K_r} -specific blocker), iPSC-CMs exhibited prolonged action potential duration (APD) and early afterdepolarizations (EADs) (Fig. 3B). Transduction of hERG 1b shRNA or the PAS fragment significantly prolonged the APD₉₀ of APs paced at 1 Hz (Fig. 3E). Additionally, transduction of either shRNA or the PAS fragment triggered EADs in cells paced at 1 Hz (Fig. 3C, D, and F), whereas EADs were not observed in control cells (Fig. 3A, B, and E and *Fig. S3*). The fractions of cells producing EADs at 1 Hz were 0.27 ± 0.02 ($n = 400$ APs from eight microclusters) and 0.32 ± 0.03 ($n = 330$ APs from seven microclusters) in iPSC-CMs transduced with shRNA and the PAS fragment, respectively. No EADs were observed in 300 paced APs recorded from six mock-transfected control microclusters and 250 paced APs from five microclusters expressing a nontargeting shRNA control vector (Fig. 3F). Similar results were observed in recordings from individual cells, indicating that the aberrant behavior arises from the cell membrane and not electrotonically among coupled cells (Fig. S3).

Beat-to-beat APD variability, compared with APD itself, is a more accurate predictor of arrhythmogenic potential in cardiac disease models (20, 21) as well as individuals with heart disease (22) and LQTS (23). We recorded 50 sequential APs paced at 1 Hz and report APD variability as the 50-beat APD₉₀ variance (Fig. 4). Compared with control cells, hERG 1b modification significantly increased APD variance by 18- and 6-fold in shRNA- and PAS-transduced iPSC-CMs, respectively (Fig. 4B and *Fig. S3*) ($P < 0.01$). The distribution of normalized APD shows that modification of native hERG 1b triggers dramatic and potentially arrhythmogenic changes in AP morphology (Fig. 4C).

Discussion

We used iPSC-CMs to determine the functional role of hERG 1b in ventricular repolarization. By introducing either hERG 1b shRNA or the 1a-specific PAS fragment, we effectively silenced the contribution of native hERG 1b to I_{K_r} and AP repolarization. Consequently, I_{K_r} was transformed, adopting properties more

Fig. 3. hERG 1b modulation prolongs iPSC-CM APD. (A) Typical AP recorded from iPSC-CM transfected with nontargeting shRNA control. Sample traces showing EADs from (B) mock-transfected control, (C) 1b shRNA-transfected, and (D) PAS-infected iPSC-CMs. The orange trace in B is from the same recording after perfusion of 12 nM E-4031. (E) APD₉₀ for control, shRNA-, and PAS-transduced iPSC-CMs during 1-Hz pacing. (F) Fractional occurrence of EADs in control, shRNA-, and PAS-transduced iPSC-CMs. All data are reported as means $\pm$ SEMs. $n = 5$ –10. (Scale bar: 40 mV $\times$ 400 ms.) *Statistical significance at $P < 0.05$.

Fig. 4. hERG 1b modulation increases APD variability. (A) Sample APs depicting beat-to-beat variability at 1-Hz pacing in nontargeting shRNA-transfected (Non), 1b shRNA-transfected, mock-transfected, and PAS-infected iPSC-CMs; 50 sequential APs are shown for each. (B) Average variance from 50 paced APs for non (35 ± 9 ms²; $n = 5$), shRNA ($3,441 \pm 1,171$ ms²; $n = 10$), mock (220 ± 93 ms²; $n = 6$), and PAS ($1,780 \pm 463$ ms²; $n = 7$) iPSC-CMs. (C) Histograms depicting the distribution of the normalized APD₉₀ for non and mock control (gray), shRNA (green), and PAS (blue) iPSC-CMs. (Scale bar: 20 mV $\times$ 200 ms.) *Statistical significance at $P < 0.05$.

characteristic of hERG 1a homotetramers. The changes in I_{K_r} were accompanied by a corresponding prolongation of APD₉₀, EADs, and APD variability, all hallmarks of cellular proarrhythmic behavior. These observations reveal a critical role for the 1b subunit in cardiac I_{K_r} -mediated repolarization.

iPSC-CMs enable the study of mechanisms of human cardiac disease in a conspecific model system. Patient-derived cell lines and genetic manipulation of generic iPSC-CMs have provided an array of disease-specific models, including LQT1 (24), LQT2 (25, 26), and LQT3 (27). In principle, patient-specific cell lines preserve the unique genetic background of the individual, including determinants of incomplete penetrance and variable expressivity of disease mutations (28). These cell lines have provided a uniquely powerful tool to explore human physiology and represent an exciting step toward patient-specific medicine.

Despite their utility in human arrhythmia models, iPSC-CMs differ from adult tissue-derived cardiomyocytes (29–33). APs recorded from iPSC-CMs display depolarized diastolic potentials, myogenic AP firing, and a reduced maximum rise rate of the AP upstroke (dV/dt_{MAX}) compared with APs from adult ventricular tissue (Fig. S2) (30–34). These attributes as well as biochemical and morphological characteristics suggest that the iPSC-CMs are at an immature developmental stage (35–39) compared with a fully developed ventricular cardiomyocyte (diastolic potential < -80 mV; $dV/dt_{MAX} = 150$ – 350 V/s) (40, 41). Thus, iPSC-CMs could prove extremely valuable as a model for the developing heart, especially for cases of intrauterine fetal death and LQTS in the young (11, 18, 42).

Our study has implications for drug safety as outlined by current initiatives calling for greater accuracy and specificity in identifying potentially proarrhythmic drugs (43). The hERG assay has been successful in preventing the release of new torsadogenic drugs but with the unintended consequence of false positives that limits the number of drugs in development (43). Although the stoichiometry of 1a and 1b subunits in native I_{K_r} channels is not currently known, hERG 1a/1b currents recorded from HEK cells transfected with equal quantities of DNA encoding hERG 1a and 1b accurately recapitulate the kinetic characteristics of native I_{K_r} (11, 13, 14). Including 1b in an updated hERG assay

would more faithfully mimic cardiac I_{Kr} and contribute to a more representative in silico cardiomyocyte model (43). As part of a more comprehensive assay that also tests for compensatory inhibition of sodium or calcium currents (43), the hERG 1a/1b assay should help shepherd more drugs safely to market. In addition, including hERG 1b in studies characterizing trafficking-defective *KCNH2* mutants and their pharmacological rescue may provide new insights into disease mechanisms and potential therapeutic treatments.

Methods

Cell Culture. Human iPSC-CMs [iCell(r) Cardiomyocytes, Cellular Dynamics International] were stored and plated per the manufacturer's instructions. HEK293 cells were cultured in six-well dishes in DMEM supplemented with 10% (vol/vol) FBS.

Transfection/Infection. Three hERG 1b-specific shRNA constructs in the pLKO.1-NEO-CMV TurboGFP vector custom generated by Sigma-Aldrich were screened for their ability to knock down 1b mRNA expressed in HEK293 cells. The custom shRNA construct (Sigma-Aldrich; sequence: CCACAACCCCTGGCTTCAT) was transfected using Lipofectamine 2000 (Life Technologies). Control cells were either mock-transfected in the absence of an expressing vector or transfected with a nontargeting shRNA pLKO.1-NEO-CMV TurboGFP control vector. For all AP recordings, iPSC-CMs were transfected with an adenoviral vector encoding $K_{ir2.1}$ in frame with GFP and/or an adenoviral PAS construct encoding the first 135 aa of the hERG 1a N terminus in frame with CFP to enable detection (15).

Quantitative PCR. Cells were washed with PBS and lysed with TRIzol reagent (Life Technologies). Lysate was combined with 200 μ L chloroform and centrifuged at $10,000 \times g$ at 4 °C for 15 min. The aqueous phase was combined with 0.5 μ L glycogen and 500 μ L isopropanol, incubated at room temperature for 10 min, and centrifuged at $10,000 \times g$ at 4 °C for 10 min. The pellet was washed with 75% ethanol, vortexed, and centrifuged for 5 min at $9,500 \times g$ at 4 °C. The pellet was then air-dried and suspended in 20 μ L diethylpyrocarbonate (DEPC)-treated H_2O . mRNA quantitative RT-PCR analysis was completed using subunit-specific primers from Invitrogen, an iScript Reverse Transcription Supermix Kit (Biorad), and an Eppendorf thermocycler per the manufacturer's instructions.

Immunocytochemistry. Immunocytochemistry was completed as previously described (17). Details are in *SI Methods*. Briefly, cells were incubated in blocking solution containing 1:200 dilutions of primary antibodies targeting hERG 1b (ALX-215-051-R100; Enzo Life Sciences) and β -actin (mAbcam 8224;

Abcam). Cells were washed and then incubated with blocking solution containing 1:1,000 dilutions of secondary antibodies Alexa Fluor 488 Goat Anti-Mouse IgG (H+L) A-11029 for β -actin and Alexa Fluor 647 Goat Anti-Rabbit IgG A-21245 (Life Technologies) for hERG 1b. Cells were viewed on a Zeiss Axiovert 200M microscope in Apotome mode with a 63 $\times$ oil immersion objective. Fluorescence intensity was measured with linear scans of individual cells using Axiovision 4.0 (Carl Zeiss Microscopy).

Electrophysiology. All recordings were conducted 10–40 d postplating and 48–60 h posttransduction at 36 ± 1 °C. Recordings were made using an Axon 200A amplifier and Clampex (Molecular Devices). Data were sampled at 10 kHz and low pass-filtered at 1 kHz. Cells were perfused with extracellular solution containing 150 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 15 mM glucose, 10 mM Hepes, and 1 mM Na-pyruvate and titrated to pH 7.4 using NaOH; 2- to 4.5-M Ω recording pipettes were backfilled with intracellular solution containing 5 mM NaCl, 150 mM KCl, 2 mM $CaCl_2$, 5 mM EGTA, 10 mM Hepes, and 5 mM MgATP and titrated to pH 7.2 using KOH (supplemented with 50 μ g/mL gramicidin D for current-clamp recordings).

All voltage protocols were completed before and after bath perfusion of 2 μ M E-4031 (an I_{Kr} -specific blocker), and the difference in current was taken to represent I_{Kr} (29). To measure I_{Kr} voltage dependence, cells were held at -40 mV to inactivate voltage-gated sodium currents and then stepped to a conditioning prepulse (-50 to $+30$ mV in 10-mV increments) for 3 s. Tail currents were measured during a subsequent 6-s step to -40 mV. I_{Kr} conductance curves were fitted with the Boltzmann equation (see *SI Methods*). The time course of I_{Kr} deactivation was measured by fitting E-4031-sensitive I_{Kr} decay at -40 mV with up to a double exponential function. Double exponential fits were reported as a weighted time constant (τ_w) to represent the fast- and slow-decay components. Spontaneous APs were recorded in gap free mode, and APs were paced at 1 Hz using a 5-ms, 400-pA stimulus.

Statistical Analysis. Analysis was completed using Clampfit (Molecular Devices) and Origin (OriginLab). All data were reported as means $\pm$ SEMs and compared using a Student *t* tests and an ANOVA, where applicable. Statistical significance was taken at $P < 0.05$.

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- Sanguinetti MC, Jiang C, Curran ME, Keating MT (1995) A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. *Cell* 81(2):299–307.
- Trudeau MC, Warmke JW, Ganetzky B, Robertson GA (1995) HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* 269(5220):92–95.
- Curran ME, et al. (1995) A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 80(5):795–803.
- Food and Drug Administration, HHS (2005) International Conference on Harmonisation; guidance on S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals; availability. Notice. *Fed Regist* 70(202):61133–61134.
- Snyders DJ, Chaudhary A (1996) High affinity open channel block by dofetilide of HERG expressed in a human cell line. *Mol Pharmacol* 49(6):949–955.
- Lees-Miller JP, Kondo C, Wang L, Duff HJ (1997) Electrophysiological characterization of an alternatively processed ERG K $^{+}$ channel in mouse and human hearts. *Circ Res* 81(5):719–726.
- London B, et al. (1997) Two isoforms of the mouse ether-a-go-go-related gene coassemble to form channels with properties similar to the rapidly activating component of the cardiac delayed rectifier K $^{+}$ current. *Circ Res* 81(5):870–878.
- Morais-Cabral JH, Robertson GA (August 23, 2014) The enigmatic cytoplasmic regions of KCNH channels. *J Mol Biol*, 10.1016/j.jmb.2014.08.008.
- Morais Cabral JH, et al. (1998) Crystal structure and functional analysis of the HERG potassium channel N terminus: A eukaryotic PAS domain. *Cell* 95(5):649–655.
- Phartiyal P, Sale H, Jones EM, Robertson GA (2008) Endoplasmic reticulum retention and rescue by heteromeric assembly regulate human ERG 1a/1b surface channel composition. *J Biol Chem* 283(7):3702–3707.
- Sale H, et al. (2008) Physiological properties of hERG 1a/1b heteromeric currents and a hERG 1b-specific mutation associated with long-QT syndrome. *Circ Res* 103(7):e81–e95.
- Abi-Gerges N, et al. (2011) hERG subunit composition determines differential drug sensitivity. *Br J Pharmacol* 164(2b):419–432.
- Larsen AP, Olesen SP (2010) Differential expression of hERG1 channel isoforms reproduces properties of native I_{Kr} and modulates cardiac action potential characteristics. *PLoS ONE* 5(2):e9021.
- McPate MJ, et al. (2009) hERG1a/1b heteromeric currents exhibit amplified attenuation of inactivation in variant 1 short QT syndrome. *Biochem Biophys Res Commun* 386(1):111–117.
- Trudeau MC, Leung LM, Roti ER, Robertson GA (2011) hERG1a N-terminal eag domain-containing polypeptides regulate homomeric hERG1b and heteromeric hERG1a/hERG1b channels: A possible mechanism for long QT syndrome. *J Gen Physiol* 138(6):581–592.
- Gustina AS, Trudeau MC (2013) The eag domain regulates hERG channel inactivation gating via a direct interaction. *J Gen Physiol* 141(2):229–241.
- Jones EM, Roti EC, Wang J, Delfosse SA, Robertson GA (2004) Cardiac I_{Kr} channels minimally comprise hERG 1a and 1b subunits. *J Biol Chem* 279(43):44690–44694.
- Crotti L, et al. (2013) Long QT syndrome-associated mutations in intrauterine fetal death. *JAMA* 309(14):1473–1482.
- Sartiani L, et al. (2007) Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: A molecular and electrophysiological approach. *Stem Cells* 25(5):1136–1144.
- Jacobson I, Carlsson L, Duker G (2011) Beat-by-beat QT interval variability, but not QT prolongation per se, predicts drug-induced torsades de pointes in the anaesthetized methoxamine-sensitized rabbit. *J Pharmacol Toxicol Methods* 63(1):40–46.
- Gallacher DJ, et al. (2007) In vivo mechanisms precipitating torsades de pointes in a canine model of drug-induced long-QT1 syndrome. *Cardiovasc Res* 76(2):247–256.
- Hinterseer M, et al. (2010) Usefulness of short-term variability of QT intervals as a predictor for electrical remodeling and proarrhythmia in patients with nonischemic heart failure. *Am J Cardiol* 106(2):216–220.
- Hinterseer M, et al. (2009) Relation of increased short-term variability of QT interval to congenital long-QT syndrome. *Am J Cardiol* 103(9):1244–1248.
- Moretti A, et al. (2010) Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med* 363(15):1397–1409.
- Matsa E, et al. (2011) Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. *Eur Heart J* 32(8):952–962.

26. Itzhaki I, et al. (2011) Modelling the long QT syndrome with induced pluripotent stem cells. *Nature* 471(7337):225–229.
27. Ma D, et al. (2013) Modeling type 3 long QT syndrome with cardiomyocytes derived from patient-specific induced pluripotent stem cells. *Int J Cardiol* 168(6):5277–5286.
28. Giudicessi JR, Ackerman MJ (2013) Determinants of incomplete penetrance and variable expressivity in heritable cardiac arrhythmia syndromes. *Transl Res* 161(1):1–14.
29. Ma J, et al. (2011) High purity human-induced pluripotent stem cell-derived cardiomyocytes: Electrophysiological properties of action potentials and ionic currents. *Am J Physiol Heart Circ Physiol* 301(5):H2006–H2017.
30. Doss MX, et al. (2012) Maximum diastolic potential of human induced pluripotent stem cell-derived cardiomyocytes depends critically on I(Kr). *PLoS ONE* 7(7):e40288.
31. Kong CW, Akar FG, Li RA (2010) Translational potential of human embryonic and induced pluripotent stem cells for myocardial repair: Insights from experimental models. *Thromb Haemost* 104(1):30–38.
32. Liang P, et al. (2013) Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 127(16):1677–1691.
33. Lieu DK, et al. (2013) Mechanism-based facilitated maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Arrhythm Electrophysiol* 6(1):191–201.
34. Zhang H, et al. (2012) Modulation of hERG potassium channel gating normalizes action potential duration prolonged by dysfunctional KCNQ1 potassium channel. *Proc Natl Acad Sci USA* 109(29):11866–11871.
35. Robertson C, Tran DD, George SC (2013) Concise review: Maturation phases of human pluripotent stem cell-derived cardiomyocytes. *Stem Cells* 31(5):829–837.
36. Jezek K, Pucelik P, Sauer J, Barták F (1982) Basic electrophysiological parameters and frequency sensitivity of the ventricular myocardium of human embryos. *Physiol Bohemoslov* 31(1):11–19.
37. Tuganowski W, Cekański A (1971) Electrical activity of a single fibre of the human embryonic heart. *Pflugers Arch* 323(1):21–26.
38. Mummery C, et al. (2003) Differentiation of human embryonic stem cells to cardiomyocytes: Role of coculture with visceral endoderm-like cells. *Circulation* 107(21):2733–2740.
39. Gennser G, Nilsson E (1970) Excitation and impulse conduction in the human fetal heart. *Acta Physiol Scand* 79(3):305–320.
40. Schram G, Pourrier M, Melnyk P, Nattel S (2002) Differential distribution of cardiac ion channel expression as a basis for regional specialization in electrical function. *Circ Res* 90(9):939–950.
41. Jost N, et al. (2013) Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. *J Physiol* 591(Pt 17):4189–4206.
42. Schwartz PJ, et al. (1998) Prolongation of the QT interval and the sudden infant death syndrome. *N Engl J Med* 338(24):1709–1714.
43. Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N (2014) Rechanneling the cardiac proarrhythmia safety paradigm: A meeting report from the Cardiac Safety Research Consortium. *Am Heart J* 167(3):292–300.