Establishment and characterization of a stable hERG cell line for high-throughput drug cardiac safety screening

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Cardiovascular safety assessment is a pivotal milestone in preclinical drug development, as drug-induced cardiovascular toxicities remain a leading cause of late-stage clinical trial failures and post-marketing withdrawals [1]. To standardize global cardiovascular safety testing, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) established the ICH S7B guideline, which mandates evaluating drug candidates for arrhythmia risk-particularly risk linked to electrocardiogram (ECG) QT interval prolongation [2]. The QT interval reflects ventricular depolarization and repolarization duration; its prolongation is a well-validated biomarker for increased risk of torsades de pointes (TdP), a life-threatening polymorphic ventricular arrhythmia [2].

The hERG potassium channel-encoded by the human Ether-à-go-go Related Gene (hERG)-is the primary mediator of cardiac ventricular repolarization, facilitating outward potassium currents (IKr) that restore the myocardial electrical gradient after each heartbeat [3]. Drug-induced hERG channel block delays repolarization, directly prolonging the QT interval and increasing drug-induced long QT syndrome risk [3]. ICH S7B strongly recommends hERG-expressing cell-based ion channel assays as a core component of nonclinical safety testing [2]; these assays enable direct measurement of drug-induced hERG inhibition, allowing researchers to prioritize compounds with low cardiotoxicity risk early in development-reducing late-stage failures and improving patient safety [2].

Traditionally, manual patch-clamp has been the “gold standard” for hERG current measurement. This technique uses glass microelectrodes to quantify electrical activity of hERG channels (in either induced hERG-expressing cells or native cardiomyocytes), offering high precision for channel gating kinetics and drug sensitivity analyses [4]. However, its reliance on specialized expertise, low throughput (1-5 compounds per day per operator), and labor-intensive workflow make it impractical for screening the thousands of candidates typical of modern drug discovery [4].

Alternative high-throughput methods (e.g., radioligand binding, thallium flux) have been proposed to address this limitation. Radioligand assays measure competitive binding of test compounds to ^{3}H-labeled dofetilide (a high-affinity hERG blocker), while thallium flux assays use thallium-based dyes to indicate intracellular potassium concentration changes [5-6]. Despite higher throughput, these methods are indirect, prone to false positives/negatives, and unable to quantify functional channel inhibition. Radioligand assays also pose safety and waste disposal challenges due to radioactive reagents, further limiting utility [5].

High-throughput automated patch-clamp (APC) systems (e.g., SyncroPatch 384i) have emerged as a transformative solution, combining patch-clamp accuracy with automation to enable screening of hundreds of compounds daily [7]. APC uses microfluidic planar chips to capture cells and form high-resistance seals, with robotic liquid handling streamlining solution exchange and

data acquisition-reducing human error and improving assay standardization [7]. A critical limitation of APC, however, is its reliance on random cell sampling (unlike manual patch-clamp, which allows selection of viable, high-expression cells). This requires cell models with uniform, high-level hERG expression to ensure sufficient valid data [7].

Transient hERG transfection has been used for APC-compatible hERG-expressing cells but suffers from variable expression (70-80% positive cells), reduced post-freeze-thaw viability, and batch-to-batch variability [8]. In contrast, stable cell lines ensure consistent, reliable hERG expression across passages and experiments-critical for reproducible, comparable data (expression fluctuations cause result variability). Additionally, stable cell lines eliminate repeated transfection and cryopreservation needs, streamlining workflows and reducing variability from these steps [8].

Here, we report the construction and characterization of a stable HEK293T (human renal epithelial) cell line with high, uniform hERG expression. We validated its functionality via manual/automated patch-clamp, confirmed sensitivity to the hERG-specific blocker E-4031 hydrochloride, and demonstrated suitability for high-throughput drug safety screening. This model addresses key limitations of existing systems, providing a reliable platform for early-stage hERG-related cardiac safety assessment.

The stable HEK293T cell line overexpressing HA-tagged hERG was generated using a lentiviral delivery system. HEK293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomycin at 37°C in 5% CO_2. The hERG gene was cloned into the lentiCas9-EGFP vector, with an HA tag fused to its N-terminus to generate the Lenti-HA-hERG-P2A-EGFP plasmid. As shown in Figure 1A, the hERG and EGFP gene fragments are separated by a P2A self-cleaving peptide-enabling co-expression of hERG and EGFP, with the final hERG protein lacking the GFP tag.

Replication-deficient lentivirus was produced by transiently transfecting 0.75 μg psPAX2, 0.25 μg pMD2.G, and 1 μg Lenti-HA-hERG-P2A-EGFP into HEK293T cells seeded in 6-well plates. Viral supernatants were collected at 48 h, filtered through a 0.45 μm filter, and diluted in fresh DMEM containing 10 μg/mL polybrene for HEK293T cell infection. At 48 hours post-infection, cells were resuspended in fresh medium, and GFP-positive cells were sorted using a CytoFLEX SRT (Beckman Coulter, USA; Figure 1A). hERG-expressing cells exhibited strong GFP signals, while empty vector-transfected control cells showed no GFP expression (Figure 1B).

To confirm hERG protein expression and subcellular localization, HEK293T cells were seeded onto 4-chamber confocal culture dishes and incubated for 24 hours at 37°C. Cells were washed with PBS, fixed in 4% paraformaldehyde (PFA), permeabilized, and blocked for 1 hour with blocking buffer (1× PBS, 5% BSA, 0.3% Triton X-100). Primary anti-HA tag antibody (Cell Signaling Technologies, 3724) and secondary goat anti-mouse IgG antibody (HUABIO, HA1006) were diluted in dilution buffer (1×PBS, 1% BSA, 0.3% Triton X-100) per manufacturer instructions and incubated sequentially at room temperature (secondary antibody in the dark for 1 hour). Nuclei were stained with DAPI, and imaging was performed using a ZEISS LSM980 laser scanning confocal microscope (image processing via Image J). As shown in Figure 1C, 293T-hERG cells displayed clear HA (red, hERG marker) and GFP (green) signals, while blank 293T cells showed negligible signals. Merged images confirmed successful hERG expression and

correct membrane localization.

Whole-cell manual patch-clamp (HEKA Elektronik, Lambrecht, Germany) was used to verify hERG channel function. Glass pipettes (Biomedical Instruments, Zollnitz, Germany) were pulled with a DMZ puller (Zeitz Instruments, Martinsried, Germany) to 0.9-2.5 MΩ and filled with intracellular solution (ICS: 140 mM KCl, 1 mM EGTA, 10 mM HEPES, 10 mM NaCl, 18 mM sucrose; pH 7.33 with KOH, osmolarity 310 mOsm with sucrose). Cells were pre-incubated in extracellular solution (ECS: 140 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 20 mM glucose, 0.2% DMSO; pH 7.4 with NaOH, osmolarity 305 mOsm with glucose) for ≥5 minutes. Leak current was subtracted online via a P/4 protocol; signals were sampled at 100 kHz and filtered at 10 kHz. After establishing whole-cell configuration (80% series resistance compensation, capacitive transient cancellation), an initial step protocol (depolarizing pulse every 10 s for 5 minutes) stabilized currents. hERG current was measured using a 2-s step protocol: depolarization to 20 mV (2 s), followed by repolarization to -40 mV (2 s), repeated every 15 s (holding potential = -80 mV). As shown in Figure 2A, traces exhibited typical hERG currents: small, stable currents during depolarization to 20 mV, and large outward tail currents (a hallmark of hERG gating) during repolarization to −40 mV, confirming normal channel function.

Peak tail current distribution was analyzed using a SyncroPatch 384i APC system (Nanion Technologies, Munich, Germany). At 70-80% confluence, stable hERG cells were harvested with TrypLE (Sigma-Aldrich, Sydney, Australia), washed with cold (4°C) FBS buffer, and resuspended in cold external solution (140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES; pH 7.4) to 1.0-1.5 × 10^6 cells/mL. Whole-cell recordings followed Nanion's protocol: cells were stored at 10°C (200 rpm shake speed) and added to a 384-well recording chamber (NPC-384, Nanion Technologies) with 1 patch aperture/well containing divalent-free solution (140 mM NaCl, 4 mM KCl, 5 mM glucose, 10 mM HEPES; pH 7.4). Fifty percent of the divalent-free solution was then exchanged with Ca^{2+}-containing seal enhancer solution (130 mM NaCl, 4 mM KCl, 10 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 10 mM HEPES; pH 7.4) to enhance seal resistance, and cells were washed twice with external solution. After establishing whole-cell configuration and recording baseline currents, compounds were added. The voltage-clamp protocol matched manual patch-clamp, and peak tail current at - 40 mV were defined as hERG current amplitude. As shown in Figure 2B and Table 1: 100% of blank 293T cells (n=48) had currents <50 pA ; of 216 hERG cells tested, 93.5% (n=202) had currents >50 pA (87% >100 pA), with 13.9% (n=30) at 100-200 pA, 23.6% (n=51) at 200-400 pA, 20.8% (n=45) at 400-600 pA, and 28.7% (n=62) >600 pA.

SyncroPatch 384i were then applied to assess the sensitivity of stable hERG cells to the hERG-specific blocker E-4031. hERG cells were treated with E-4031 (27.5, 82.5, 247.5, 742.5 nM) or DMSO (control); peak tail currents were recorded over time. Whole-cell data were filtered by the criteria: seal resistance >500 MΩ, series resistance <15 MΩ, cell capacitance 3-50 pF, baseline peak current >100 pA. As shown in Figure 2C, E-4031 induced concentration-dependent current reduction, confirming dose-dependent hERG block. Figure 2D shows the normalized E-4031 concentration-response curve. % block was calculated as [(control - drug)/control] × 100. Half-maximal inhibitory concentration (IC_{50}) was fit to a 4-parameter Hill equation (GraphPad Prism, constrained bottom=0, top=1) and determined to be 29.8 nM (n=14), which is consistent with literature values [9], validating the cell line's physiological relevance.

In this study, we successfully established a stable HEK293T cell line with high hERG

expression and canonical channel function, ideal for APC-based high-throughput screening. Lenti-HA-hERG-P2A-EGFP plasmid construction, lentiviral infection, and flow cytometric sorting ensured efficient, specific hERG expression-confirmed by confocal microscopy. Functional validation via manual/APC patch-clamp demonstrated typical hERG currents: manual patch-clamp revealed characteristic voltage-dependent current patterns, while SyncroPatch 384i showed 93% of hERG cells had peak tail currents >50 pA (indicating robust function). Pharmacological testing with E-4031 yielded a concentration-dependent block and IC_{50} of 29.8 nM (matching literature [9]), confirming the cell line's reliability for hERG inhibition assays.

This stable cell line addresses limitations of traditional manual patch-clamp (low throughput, labor intensity) and transient transfection (variable expression, poor post-freeze-thaw viability). Its consistent hERG expression enhances data reproducibility, which is critical for high-throughput screening, as it enables efficient evaluation of drug candidates for hERG block and QT prolongation risk. By combining stable hERG expression with APC efficiency, this platform facilitates early detection of hERG-related cardiac safety risks, guiding drug candidate optimization to reduce cardiotoxicity and improve clinical trial success rates-ultimately supporting safer, more effective medication development. Future work may explore its application in screening diverse chemical libraries and investigating mechanisms of hERG channel modulation.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Table 1 Current distributions of stable HEK293T-hERG cells detected by APC system

Amplitute (nA)	＜0.05	0.05-0.1	0.1-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1	＞1
Number	14	14	30	51	45	30	16	16
Ratio (%)	6.5	6.5	13.9	23.6	20.8	13.9	7.4	7.4

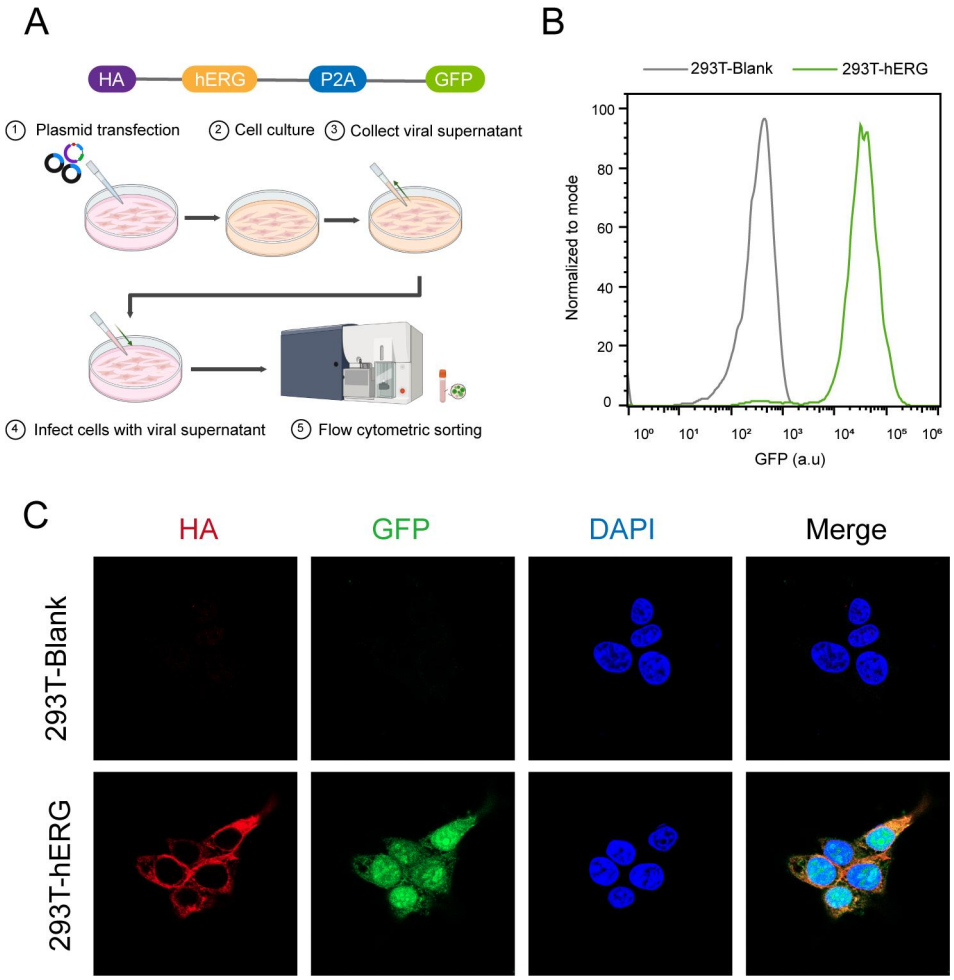

Figure 1. Generation of HEK293T-hERG stable cell line. (A) Schematic illustration of the workflow for generating HEK293T-hERG stable cell line, including plasmid transfection, cell culture, viral supernatant collection, cell infection, and flow cytometric sorting. The plasmid is designed to co-express HA-tagged hERG and GFP, with the P2A self-cleaving peptide enabling the separation of the two proteins after translation. (B) Flow cytometry analysis of GFP expression in 293T-blank and 293T-hERG cells. The green peak represents HEK293T-hERG cells with high GFP expression, while the gray peak represents 293T-blank cells with negligible GFP expression. (C) Confocal microscopy images showing the expression and localization of HA (red, labeling hERG) and GFP (green) in blank and hERG cells. DAPI (blue) stains the nuclei.

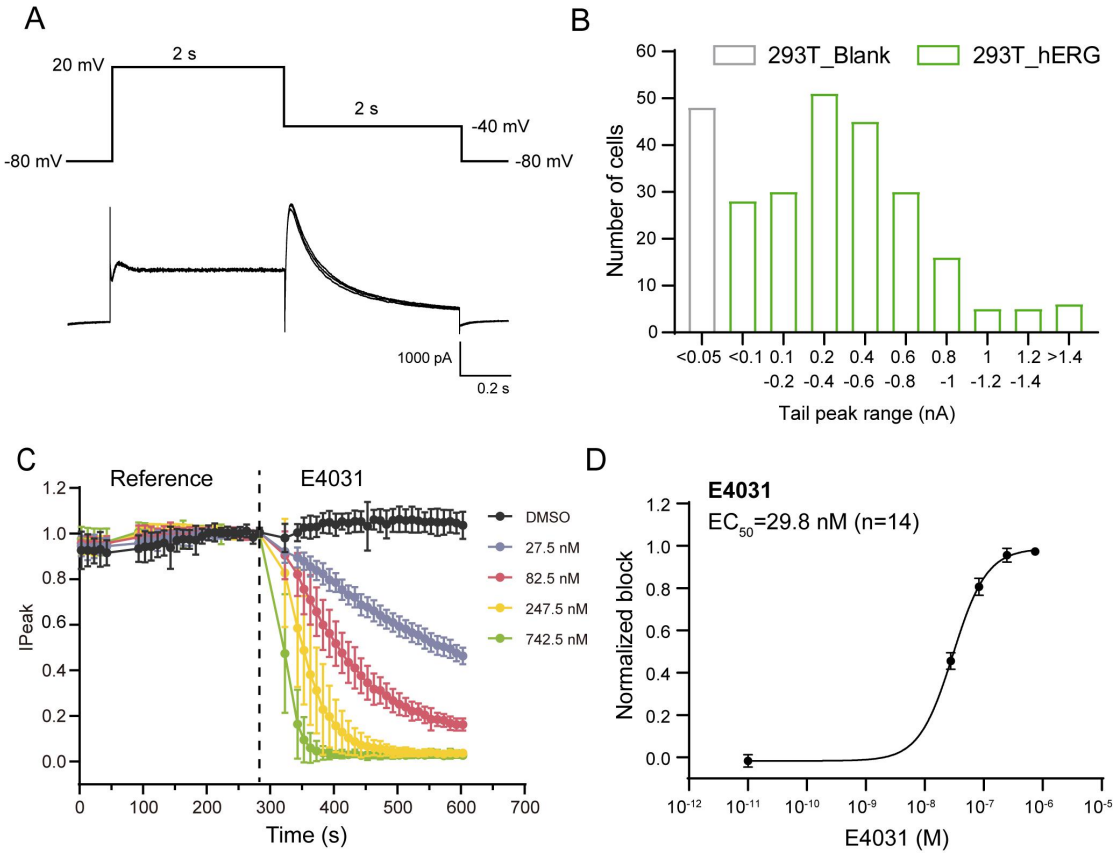

Figure 2. Function characterization of hERG channels in HEK293T cells. (A) Representative current traces of hERG channels in stable hERG cells recorded via manual patch-clamp. The upper part shows the voltage protocol. The lower part displays the corresponding hERG currents, with the scale bar indicating 1000 pA for current and 0.2 s for time. (B) Distribution of tail peak currents in blank and hERG cells detected by APC. The histogram illustrates the number of cells within different tail peak current ranges (nA), where blank cells (gray bars) mainly have tail peak currents less than 0.05 nA, while hERG cells (green bars) exhibit a wide range of larger tail peak currents. (C) Time - course of peak current inhibition by different concentrations of E-4031 (27.5 nM, 82.5 nM, 247.5 nM, 742.5 nM) and DMSO control. (D) Concentration-response curve for E-4031-mediated inhibition of hERG channels, with the half-maximal effective concentration (EC_{50}) calculated to be 29.8 nM (n = 14). Error bars represent standard deviation.