Functional characteristics of human iPSC-derived cardiomyocytes as in-vitro disease model to study drug effects

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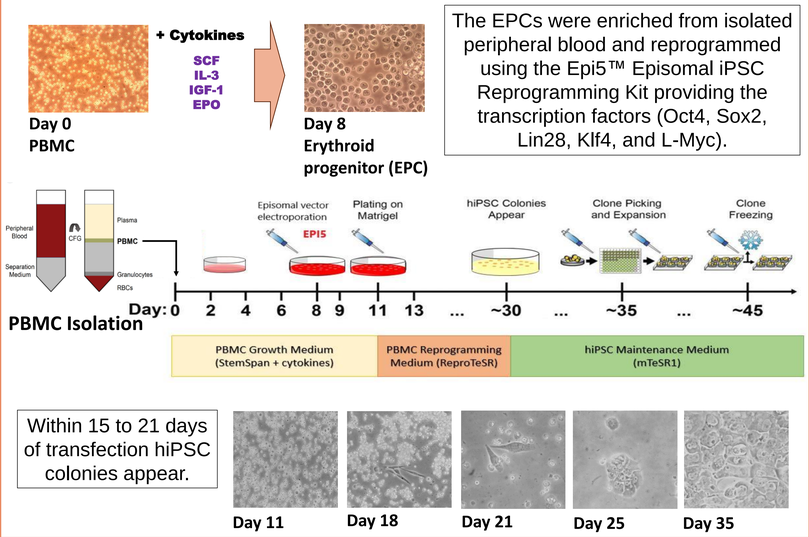
Background

- Human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (-CMs) offer vast potential for human disease modelling, regeneration, physiology and pharmacology research (Li et al., Int J Mol Sci., 2020).
- hIPSC-CM have previously been employed to study the electrophysiological abnormalities in patients with various inherited arrhythmia syndromes, including long-QT syndrome (LQTS, Pourrier and Fedida, Int J Mol Sci., 2020). However, the electrophysiological properties of hIPSC-CM generated at CARIM have not previously been characterized.
- To ultimately study arrhythmogenic mechanisms in a patient with long-QT syndrome type 3, related to dysfunction of the cardiac Na^+ channel, we generated three human-derived hiPSC lines (H1/CARIMi008 of the LQTS patient, T5/CARIMi009 of the patient's sister who does not carry the LQTS-associated genetic mutation, and an independent control S1/CARIMi007) and characterized their baseline electrophysiological properties and response to drugs.

Methods

- hiPSCs were generated from erythroid progenitor cells expanded out of peripheral blood mononuclear cells (PBMCs; Fig. 1).
- Expanded cells were reprogrammed using the Neon electroporation system and oriP/EBNA-1 backbone episomal vectors kit Epi5 (Fig. 1).
- Suitable hiPSC clones were characterized (Fig. 2). and following cardiac differentiation, maturated hiPSC-CMs were generated (Fig. 3).
- Action potentials (APs) were recorded at 37°C using the current-clamp technique, a simulated I_{K1} current was injected in real time using dynamic clamp to compensate for the low I_{K1} expression in hIPSC-CM and normalize the resting membrane potential (RMP, Fig. 4).
- I_{Na} and tetrodotoxin (TTX)-sensitive I_{NaL} currents were measured at room temperature using the whole-cell voltage-clamp method (Fig. 5).

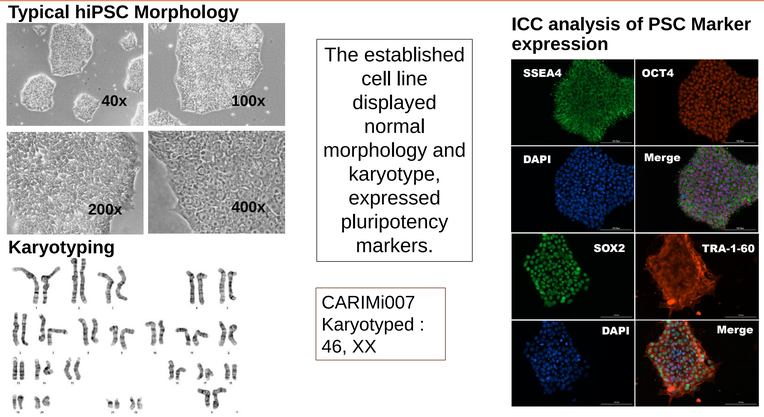
Figure 1: Generation of hiPSCs pipeline



The EPCs were enriched from isolated peripheral blood and reprogrammed using the Epi5™ Episomal iPSC Reprogramming Kit providing the transcription factors (Oct4, Sox2, Lin28, Klf4, and L-Myc).

Within 15 to 21 days of transfection hiPSC colonies appear.

Figure 2: Characterization of CARIMi007 hiPSCs



The established cell line displayed normal morphology and karyotype, expressed pluripotency markers.

CARIMi007 Karyotyped : 46, XX

Figure 3: Schematic of subsequent hiPSC-CMs generation

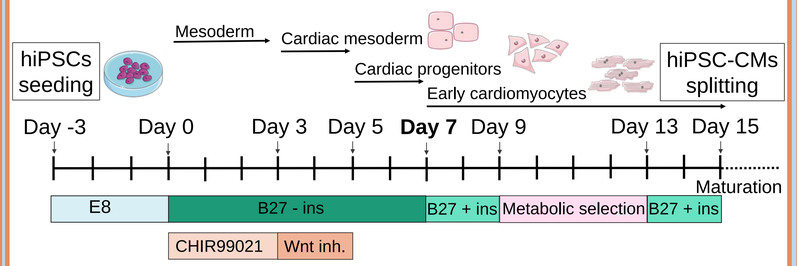
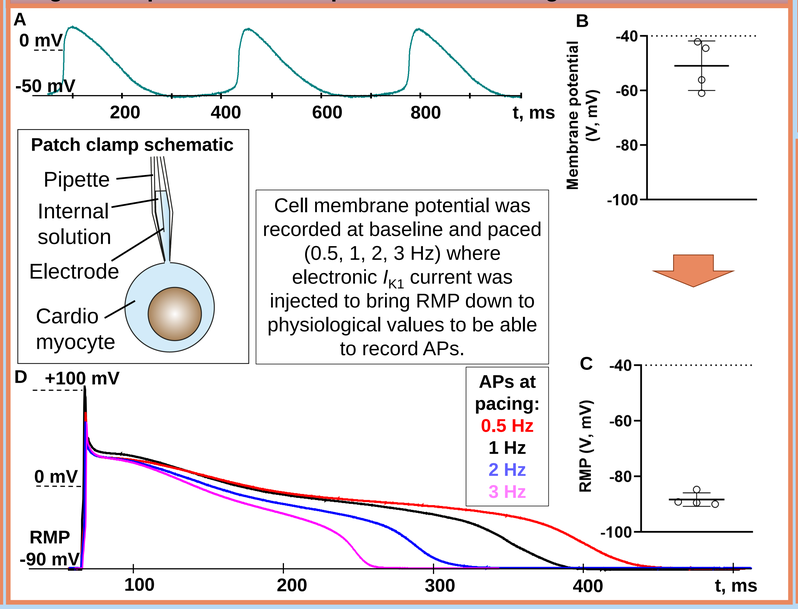
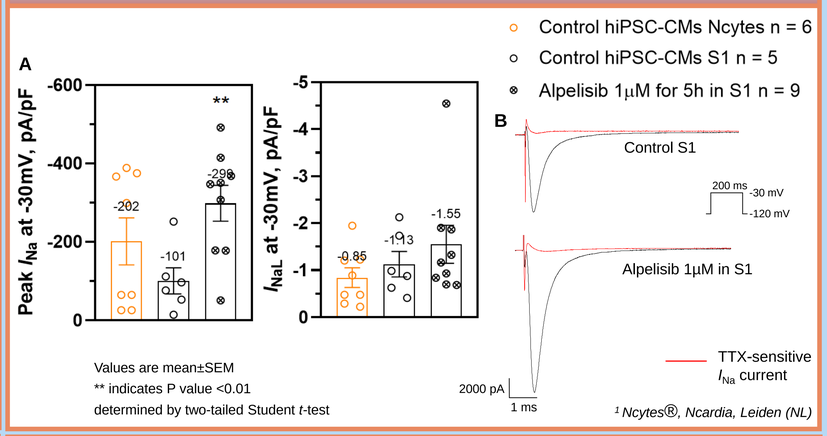


Figure 4: Spontaneous and paced APs recordings of CARIMi007



Cell membrane potential was recorded at baseline and paced (0.5, 1, 2, 3 Hz) where electronic I_{K1} current was injected to bring RMP down to physiological values to be able to record APs.

Figure 5: Na^+ current characterization of CARIMi007



[1] Ncytes®, Ncardia, Leiden (NL)

Results

- hiPSCs were generated from 3 patients. Representative data from CARIMi007 are presented in Fig. 2, 4, 5. Cells exhibited typical hiPSCs morphology and pluripotency markers, had no chromosomal aberrations (Fig. 2).
- Following cardiac differentiation, spontaneous beating hiPSC-CMs were observed by day 7 (Fig. 3).
- Differentiated and maturated unpaced CARIMi007 hiPSC-CMs had a membrane potential of -50.9±9.1 mV (n=4; Fig. 4A, B). Dynamic I_{K1} injection lowered it to -88.4±2.4 mV (Fig. 4C), enabling recording of paced APs (Fig. 4D). CARIMi007 hiPSC-CMs exhibited the expected negative cycle length dependence of AP duration (Fig. 4D)
- I_{Na} parameters in CARIMi007 hIPSC-CMs were similar to commercially available Ncytes[1] hiPSC-CMs (Fig. 5A).
- Finally, application of the anti-cancer drug alpelisib for 5 hours showed the expected peak I_{Na} increase and a trend towards I_{NaL} growth in CARIMi007 hIPSC-CMs (Fig. 5A, B).

[1] Ncytes®, Ncardia, Leiden (NL)

Conclusions

- In-house reprogramming of blood cells enabled successful generation of three hiPSC cell lines, as confirmed by morphology, karyotype and expression of pluripotency markers.
- Cardiac differentiation resulted in spontaneously beating hiPSC-CMs of a mixed electrophysiological phenotype including atrial, ventricular and nodal characteristics with physiological APD rate dependence after injection of I_{K1} with dynamic clamp.
- In-house generated control hiPSC-CMs displayed I_{Na} properties comparable to commercial hiPSC-CMs and enabled characterization of drug-induced I_{Na} regulation, which may contribute to the initiation of cardiac arrhythmias in predisposed individuals.

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