Developmental changes in electrophysiological characteristics of human-induced pluripotent stem cell–derived cardiomyocytes

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BACKGROUND Previous studies proposed that throughout differentiation of human induced Pluripotent Stem Cell–derived cardiomyocytes (iPSC-CMs), only 3 types of action potentials (APs) exist: nodal-, atrial-, and ventricular-like.

OBJECTIVES To investigate whether there are precisely 3 phenotypes or a continuum exists among them, we tested 2 hypotheses: (1) During culture development a cardiac precursor cell is present that—depending on age—can evolve into the 3 phenotypes. (2) The predominant pattern is early prevalence of a nodal phenotype, transient appearance of an atrial phenotype, evolution to a ventricular phenotype, and persistence of transitional phenotypes.

METHODS To test these hypotheses, we (1) performed fluorescence-activated cell sorting analysis of nodal, atrial, and ventricular markers; (2) recorded APs from 280 7- to 95-day-old iPSC-CMs; and (3) analyzed AP characteristics.

RESULTS The major findings were as follows: (1) fluorescence-activated cell sorting analysis of 30- and 60-day-old cultures showed that an iPSC-CMs population shifts from the nodal to the atrial/ventricular phenotype while including significant transitional populations; (2) the AP population did not consist of 3 phenotypes; (3) culture aging was associated with a shift from nodal to ventricular dominance, with a transient (57–70 days) appearance of the atrial phenotype; and (4) beat rate variability was more prominent in nodal than in ventricular cardiomyocytes, while pacemaker current density increased in older cultures.

CONCLUSION From the onset of development in culture, the iPSC-CMs population includes nodal, atrial, and ventricular APs and a broad spectrum of transitional phenotypes. The most readily distinguishable phenotype is atrial, which appears only transiently yet dominates at 57–70 days of evolution.

KEYWORDS iPSC-CMs; Action potential; Development; Beat rate variability

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Introduction

Currently available preparations of induced Pluripotent Stem Cell–derived cardiomyocytes (iPSC-CMs) include myocytes with nodal, atrial, and ventricular action potential (AP) properties,[1,2] thus providing the opportunity for developing stem cell–based therapies.[3] Several groups investigated the electrophysiological characteristics of human iPSC-CMs 5–70 days postplating and reported only 3 phenotypes: nodal, atrial, and ventricular.[1,2] The underlying assumption of this categorization appears to have been that only 3 phenotypes exist, even in cardiomyocytes studied shortly after beating begins. The assumption underlying this fundamental finding suggests a genetic predetermination of myocyte phenotype that is programmed to occur throughout the time in culture rather than continued maturation of a parent cell having transitional characteristics among the 3 phenotypes. Because this assumption has not been tested, changes in AP characteristics occurring as culture age increases were not determined and the abiding concept is that the rigid 3-phenotype electrophysiological classification is maintained throughout the time in culture. With that in mind we tested 2 hypotheses: (1) During culture development a cardiac precursor cell is present that—depending on age and conditions in culture—

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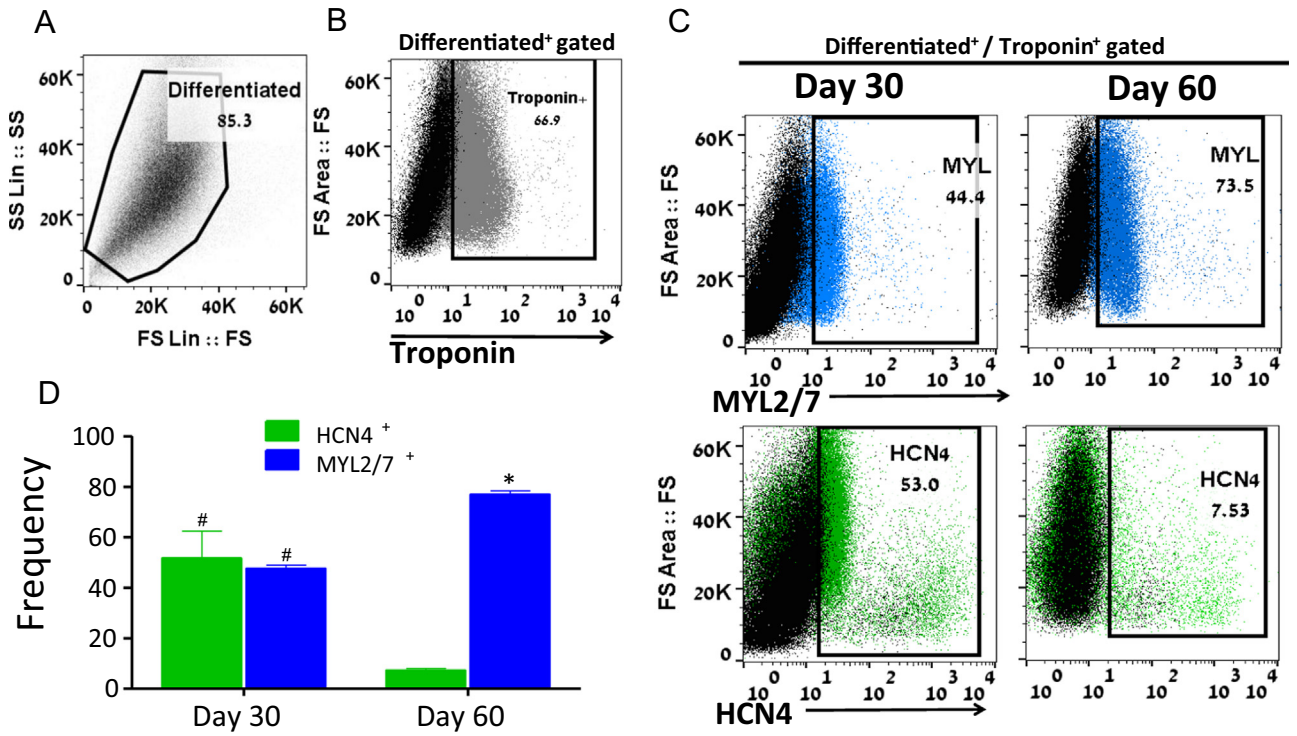

Figure 1 Fluorescence-activated cell sorting analysis of cardiac markers on 30- and 60-day-old induced Pluripotent Stem Cell-derived cardiomyocytes (iPSC-CMs). **A:** Differentiated iPSCs were gated (differentiated$^+$) according to their forward and side scatter (FS and SS, respectively) properties. **B:** Analysis of differentiated$^+$ gated cells. Cells were stained with a secondary antibody as control (black dots) or with both troponin T and a secondary antibody (gray dots). Troponin$^+$ cells were gated according to their relative fluorescence intensity (horizontal axis) and FS properties. **C and D:** Representative (panel C) and analysis (panel D) of troponin$^+$ cells relative fluorescence intensity of markers for myosin light chain (MYL2) (C, blue dots; D, blue bars), myosin light chain 7 (MYL7) (C, blue dots; D, blue bars), and hyperpolarization-activated cyclic nucleotide-gated channel (HCN4) (C, green dots; D, green bars) as atrial, ventricular, and nodal markers, respectively. The frequencies of MYL2/7 and HCN4 were compared on day 30 (C, left panels) and day 60 (C, right panels). Each analysis contained 1×10^6 differentiated cells; 4 sets of individual experiments were analyzed. $^*P < .001$, day 60 MYL2/7$^+$ vs HCN4$^+$. For both MYL2/7$^+$ and HCN4$^+$, $^\#P < .001$, day 30 vs day 60.

can evolve into nodal, atrial, or ventricular phenotypes. (2) The predominant pattern in culture is early prevalence of a nodal phenotype, transient appearance of an atrial phenotype, and ultimate evolution to a ventricular phenotype. Throughout this spectrum, the transitional phenotype persists.

Methods

iPSC-CMs generation from hair keratinocytes

iPSC-CMs were generated from keratinocytes[4] (Online Supplement) from 2 healthy women (age 39 and 54 years).

AP recording and analysis

AP and the pacemaker current (I_f) were recorded and analyzed as described in the Online Supplement (Methods, Results, and Figure 1).

Beat rate variability

APs were analyzed to determine beat rate variability (BRV) (Online Supplement).

Fluorescence-activated cell sorting

iPSC-CMs were stained with antibodies for pacemaker/nodal, ventricular, and atrial markers (Online Supplement).

Statistical analysis

See the Online Supplement.

Results

Developmental changes in cardiac markers

We investigated in 30- and 60-day-old iPSC-CMs the cardiac markers for pacemaker/nodal, ventricular, and atrial populations by staining with antibodies for troponin T, hyperpolarization-activated cyclic nucleotide-gated channel potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4), myosin light chain 2 (MYL2), and myosin light chain 7 (MYL7), respectively.[5–7] As control, we stained with a secondary antibody (Figure 1B, black dots). The representative experiment showed that (1) on days 30 (Figure 1C, left panels) and 60 (Figure 1C, right panels), cardiomyocytes represented by troponin$^+$ cells (Figure 1B, gray dots within the gate) include 2 major populations: ventricular/atrial (MYL2/7$^+$) and pacemaker/nodal (HCN4$^+$) cells (Figure 1C, blue and green dots, respectively). (2) While on day 30 the frequencies of both populations were similar (Figure 1C, left panels), on day 60 (Figure 1C, right panels) the frequency of atrial/ventricular cells was 74% vs 8% for the frequency of nodal/pacemaker cells ($P < .001$). Similar results are depicted in Figure 1D, summarizing 4 identical experiments. Next, we dot-plotted