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Developmental changes in electrophysiological characteristics of human induced Pluripotent Stem Cell-derived cardiomyocytes

Meital Ben-Ari, M.Sc^{a,b,c,*}, Shulamit Naor, M.Sc^{a,b,c,*}, Naama Zeevi-Levin, Ph.D^c, Revital Schick, M.Sc^{a,b,c}, Ronen Ben Jehuda, M.Sc^{a,b,c,d}, Irina Reiter, M.Sc^{a,b,c}, Amit Raveh, B.Sc^e, Inna Grijnevitch, B.Sc^e, Omri Barak, Ph.D^c, Michael R. Rosen, M.D^f, Amir Weissman, M.D^{c,g}, and Ofer Binah, Ph.D^{a,b,c}

^aDepartment of Physiology, Technion, Haifa, Israel

^bThe Rappaport Institute, Technion, Haifa, Israel

^cRappaport Faculty of Medicine, Technion, Haifa, Israel

^dDepartment of Biotechnology, Technion, Haifa, Israel

^eFaculty of Electrical Engineering, Technion, Haifa, Israel

^fDepartments of Pharmacology and Pediatrics, Columbia University, New York, NY, USA

^gDepartment of Obstetrics and Gynecology, Rambam, Haifa, Israel

Abstract

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

Objective—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 nodal phenotype, transient appearance of atrial phenotype, evolution to ventricular phenotype, and persistence of transitional phenotypes.

Methods—To test these hypotheses we: (1) performed FACS analysis of nodal, atrial and ventricular markers; (2) recorded AP from 280 7-to-95 day old iPSC-CMs; (3) analyzed AP characteristics.

Results—The major findings were: (1) FACS analysis of 30 and 60-day old cultures showed that an iPSC-CMs population shifts from nodal into atrial/ventricular phenotype, while including

Corresponding author: Ofer Binah, PhD, Department of Physiology, Rappaport Faculty of Medicine, POB 9649, Haifa, 31096 Israel, binah@tx.technion.ac.il; Tel: +972-4-8295262; Fax: +972-4-8513919.

*Equal contributors.

Conflict of Interest

None declared.

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significant transitional populations. (2) The AP population did not consist of 3 distinct phenotypes; (3) Culture aging was associated with a shift from nodal to ventricular dominance, with a transient (57–70 days) appearance of atrial phenotype; (4) Beat Rate Variability was more prominent in nodal than ventricular cardiomyocytes while I_f density increased in older cultures.

Conclusions—From the onset of development the iPSC-CMs population includes nodal, atrial and ventricular AP 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

iPSCs-CM; Action potential; Development; Beat Rate Variability

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-to-70 days post-plating, and reported only 3 distinct phenotypes: nodal, atrial and ventricular.^{1,2} The underlying assumption of this categorization appears to have been that only 3 distinct 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 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 - 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

iPSCs generation from hair keratinocytes

iPSC-CMs were generated from keratinocytes⁴ (On-line Supplement) from 2 healthy females (39 and 54-year old).

Action potential recording and analysis

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

Beat Rate Variability (BRV)

AP were analyzed to determine (BRV) (Online Supplement).

Fluorescence Activated Cell Sorting (FACS)

iPSC-CMs were stained with antibodies for pacemaker/nodal, ventricular and atrial markers. See On line supplement for details.

Statistical analysis

See On-line Supplement.

Results

Development changes in cardiac markers

We investigated in 30 and 60 day-old iPSC-CMs cardiac markers for pacemaker/nodal, ventricular and atrial populations, by staining with antibodies for troponin T, HCN4, myosin light chain 2 (MYL2) and myosin light chain 7 (MYL7), respectively.⁵⁻⁷ As control, we stained with a secondary antibody (Fig. 1B, black dots). The representative experiment showed that: (1) on days 30 (Fig. 1C, left panels) and 60 (Fig. 1C, right panels) cardiomyocytes represented by troponin⁺ cells (Fig. 1B gray dots within gate) include 2 major populations: ventricular/atrial (MYL2/7⁺) and pacemaker/nodal (HCN4⁺) cells (Fig. 1C, blue and green dots, respectively). (2) While on day 30 the frequencies of both populations were similar (Fig. 1C, left panels), on day 60 (Fig. 1C, right panels) the atrial/ventricular frequency was 74% versus 8% of nodal/pacemaker ($P < 0.001$). Similar results are depicted in Fig. 1D summarizing 4 identical experiments. Next we dot-plotted the expression of HCN4 and MYL2/7 markers in cardiomyocytes (Fig. 2A). Analyzing HCN4 (Fig. 2A horizontal axis) and MYL2/7 (Fig. 2A vertical axis) co-expression patterns yielded 5 subpopulations (Fig. 2A): (i) High HCN4 expression, MYL2/7⁺ (orange dots); (ii) Low HCN4 expression, MYL2/7⁺ (red dots); (iii) HCN4⁺/MYL2/7⁻ (green dots); (iv) MYL2/7⁺/HCN4⁻ (blue dots); (v) HCN4⁻/MYL2/7⁻ (black dots). Between day 30 (Fig. 2A upper panel, Fig. 2B black columns) and 60 (Fig. 2A lower panel, 2B gray columns) we observed: (1) decreased frequency of nodal/pacemaker “HCN4⁺, MYL2/7⁻” cells (green dots), “Low HCN4, MYL2/7⁺” cells (red dots) and “hiHCN4, MYL2/7⁺” cells (orange dots) (Fig. 2B, 32.5% vs. 0.8%; 24.7% vs. 3.1%; 3.0% vs. 1.8% decrease, respectively). (2) Increased frequency of atrial/ventricular cells (Fig. 2B, “MYL2/7⁺, HCN4⁻”, blue dots) (16.3% vs. 73.0%). In summary, in 30 and 60-day old iPSC-CMs the population shifts from nodal into atrial/ventricular phenotype, while including significant transitional populations.

Developmental changes in AP characteristics

Phenotypic diversity of APs—Supporting the first hypothesis, APs population appears as a heterogeneous ensemble, with no segregation into 3 distinct groups (Fig. 3A), yet includes nodal (short duration, no plateau), ventricular (long duration, prominent plateau) and atrial (sharp phase 1) phenotypes (Fig. 3B). To support this important observation of phenotypic continuity, the age range was divided into 4 groups: 1) 7–24; 2) 25–56; 3) 57–70; 4) 71–95 days (Fig 3C, D, E, F). The reason for defining the 3rd group was the appearance of

atrial AP (Fig. 3B, E) on day 57 and disappearance on day 71. A continuum of phenotypes is demonstrated in the first two age groups (Fig. 3C, D), which depict a wide spectrum of phenotypes. With further aging (Fig. 3F), AP prolongation was evident, supporting the progressive differentiation into the ventricular phenotype, concomitant with a decrease in nodal AP.

Developmental changes in electrophysiological characteristics—In support of the phenotypic continuity, we found that major AP characteristics are widely scattered, implying there are no 3 discrete clusters (Fig. 4A–E). A comparison of these AP characteristics (Fig. 4F–J) shows that amplitude ($P<0.05$, Fig. 4F), overshoot ($p<0.05$, Fig. 4G) and APD_{90} ($P<0.05$, Fig. 4H) were largest in the 4th age group. Similar to APD_{90} , APD_{20} and APD_{50} (On-line Supplement Results, Fig. 2A–B) are also scattered throughout the age groups. Finally, in contrast to other characteristics, maximal diastolic potential (MDP) was similar in all groups (Fig. 4E, J). These observations agree with the notion of iPSC-CMs differentiation into ventricular cardiomyocytes at days 71-to-95. In the 3rd group (compared to other groups), the increase in amplitude ($P<0.05$, Fig. 4F), overshoot ($P<0.05$, Fig. 4G) and dV/dt_{max} ($P<0.05$, Fig. 4I), and the decrease in APD_{90} ($P<0.05$, Fig. 4H) - all result from appearance of atrial phenotype.

Can we use APD_{90} and dV/dt_{max} to separate AP into the 3 groups?

To strengthen the conclusions on the lack of 3 separate groups, we performed a Gaussian fit (see On-line Supplement for details) to identify population clusters among the phenotypes, using APD_{90} and dV/dt_{max} (which are phenotypic dependent^{1,2,8,9}). Concerning APD_{90} in the first 2 groups (Fig. 5A–B) only one population exists, covering a wide range of APD_{90} values. APD_{90} values of these groups were 206 ± 9 and 236 ± 12 msec, respectively. The 3rd group (Fig. 5C) could be separated into: (i) a larger population with a shorter APD_{90} (159 ± 5 msec) composed mainly of nodal and atrial cells (which cannot be distinguished based on APD_{90}); (ii) a second population with longer APD_{90} (431 ± 20 msec) including mainly transitional/ventricular cells. The 4th group (Fig. 5D) also includes 2 populations: the nodal/transitional ($APD_{90} = 333\pm19$ msec) and the ventricular cells with $APD_{90} = 724\pm18$ msec. Similarly, dV/dt_{max} could not be used to generate 3 distinct groups (On-line Supplement Results and Fig. 3A–D).

Can the combination of APD_{90} and dV/dt_{max} separate the AP phenotypes?

Since neither APD_{90} nor dV/dt_{max} alone could generate 3 electrophysiological clusters, we tested whether the combination of APD_{90} and dV/dt_{max} is more effective (Fig. 5E). The figure shows that in the 2 younger age groups APD_{90} and dV/dt_{max} have lower values and exhibit compact clusters of points. With aging, APD_{90} and dV/dt_{max} become higher concomitantly with marked dispersion of the points. In conclusion, this analysis demonstrates: (1) phenotype maturation and diversity change with aging, (2) a developmental shift in iPSC-CMs phenotype, from nodal to atrial in mid-development, and then to typical ventricular in older cells.

I_f characteristics in young and old cardiomyocytes

Because all cardiomyocytes studied fired spontaneously, we determined whether aging is associated with maturation of the major pacemaker current I_f. Fig. 3G–I depicts in the young (30–35 days) and old group (63–95 days) I_f traces and mean I–V relations. These relations and the activation voltages (young: -58.6 ± 4.6 mV; old: -59.6 ± 2.4 mV, $P > 0.05$) show that while I_f activation voltage is similar in these groups, I_f current density is greater in the old group.

Analysis of Beat Rate Variability (BRV)

Since all iPSC-CMs studied fire spontaneously (Fig. 3B), we investigated whether the different phenotypes can be distinguished by their BRV characteristics¹⁰ representing their dynamic firing patterns. The first step was to illustrate BRV properties in the nodal (lack of phase 1 spike and $APD_{90} < 215$ msec, 179 ± 15 msec), ventricular ($APD_{90} > 275$ msec, 418 ± 37 msec) and atrial (clear phase 1 and a narrow range of $APD_{90} = 139 \pm 4$ msec) (Fig. 6A–C) phenotypes. The analysis included 56–95-day old cells. As illustrated by the superimposed IBIs versus time (Fig. 6D) and Poincaré plots (Fig. 6E), IBIs dispersion is much larger in the nodal phenotype, intermediate in ventricular and smaller in the atrial. However, because the mean firing rates of the entire (before the rate-based selection) nodal and ventricular cell populations were much lower (81.1 ± 6.1 and 28.4 ± 2.5 beats/min, respectively, $P < 0.05$) than the atrial (138.0 ± 4.9 beat/min), these findings cannot be interpreted to mean that the atrial phenotype has inherently lower BRV than the ventricular/nodal phenotypes. We state this because the Coefficient of Variation (COV, a common measure of BRV¹⁰) is markedly dependent on the beat rate (Fig. 6F, G). This necessitates that comparisons be made only between cells firing at similar rates. Consequently, because of their rapid rate, atrial cells were not compared to nodal/ventricular cells.

To compare the firing patterns of nodal versus ventricular phenotypes, we analyzed BRV characteristic in the respective populations firing at the same mean rates of 43 beats/min (42.7 ± 3.9 in nodal and 42.6 ± 3.2 in ventricular cells) (Fig. 6H). Importantly, as illustrated in Fig. 6I–6K, all 3 BRV characteristics were larger ($P < 0.05$) in nodal than ventricular cells. This key finding implies that nodal and ventricular cells can be distinguished based on their dynamic firing characteristics.

The development-dependent electrophysiological profile

Based on the electrophysiological findings we generated a development profile of the AP phenotypes. Although APD_{90} could not be used to generate 3 phenotypic groups, we used this measure to arbitrarily define 3 separate groups of nodal ($APD_{90} < 215$ msec, lack of phase 1 spike) and ventricular ($APD_{90} > 275$ msec, lack of phase 1 spike) action potentials. The atrial group was selected based on a clear phase 1. While the nodal phenotype decreased with age (Fig. 7A), the ventricular profile was biphasic; it increased from the 1st to the 2nd age range, decreased in the 3rd range, and then increased again in the 4th. Interestingly, the decrease in the ventricular phenotype at the 3rd age range was paralleled by a transient appearance of the atrial phenotype. The bar graph also includes transitional action potentials, not fitting any of the definitions. A schematic model explaining this complex profile is proposed in the Discussion (Fig. 7B).

Discussion

In support of our hypotheses, the major findings were: (1) FACS analysis showed that in the course of development, the iPSC-CMs population shifted from a nodal-like into an atrial/ventricular-like phenotype, and also included transitional populations. (2) Based on key AP characteristics, the entire population at different culture age ranges could not be separated into 3 distinct groups of phenotypes; (3) In the range of 7-to-95 days, we found a shift from nodal phenotype dominance in young cultures to ventricular dominance in older cultures, along with a transient (57–70 days) appearance of the atrial phenotype. (4) An increase in I_f density occurs in proceeding from the young, predominantly nodal population to the older, predominantly ventricular population.

FACS analysis of cardiac markers

The FACS analysis demonstrated several troponin⁺ subpopulations distinguished by their different expression of HCN4 (pacemaker marker), MYL7 (atrial marker) and MYL2 (ventricular marker). HCN4 (the most expressed isoform in the adult sinus node^{11,12}) expression begins early in development, continues during the generation of the cardiac conduction system and is expressed throughout maturation.⁵ Ventricular iPSC-CMs were detected by the expression of MYL2,⁶ restricted to ventricular regions in murine model, from early stages of development.¹³ Atrial iPSC-CMs were detected by MYL7 expression; although MYL7 is not restricted to atrial cardiomyocytes in the early stages of development,⁷ later on MYL7 becomes restricted to the atria.^{6,7} Due to its expression in both cell types in early development, MYL7-positive cells represent atrial and/or ventricular cardiomyocytes.

As shown in Figs. 1C and 1D, the cardiac population could be divided into 2 groups according to their HCN4 and MYL2/7 expression. Further, while on day 30 the frequencies of the nodal/pacemaker and atrial/ventricular population were similar, on day 60 the frequency of the latter was larger. Next, dot-plotting the expression of HCN4 and MYL2/7 within each time point revealed that the cardiomyocytes population consists of subpopulations which include HCN4 expressing cells exclusively (Fig. 2A, HCN4⁺, MYL2/7⁻), MYL2/7 expressing cells exclusively (Fig. 2A, HCN4⁻, MYL2/7⁺) and a transitional population which expresses both MYL2/7 and HCN4 at different intensities (Fig. 2A, low and high HCN4, MYL2/7⁺). Finally, we discovered a population of cardiomyocytes expressing neither HCN4 nor MYL2/7 (Fig. 2A, HCN4⁻, MYL2/7⁻). In agreement with Fig. 1, the frequency of the subpopulations with nodal properties diminished with age (Fig. 2B, low and high HCN4⁺, MYL2/7⁺ and HCN4⁺, MYL2/7⁻) while the frequency of the atrial/ventricular cardiomyocyte subpopulation expressing MYL2/7, increased from 16% to 73% (Fig. 2B). In summary, we demonstrated that the composition of cardiomyocyte population shifts with time in culture from nodal cells to atrial/ventricular cardiomyocytes, in agreement with a previous report in hESC-CMs showing a marked decrease in HCN4 expression between 15–40 days and 50–110 days in culture.¹⁴ These observations regarding HCN4, coupled with the observation that I_f increases between cultures having ventricular versus nodal phenotype predominance are consistent with an impact of the HCN4 on the pacemaker current.

Can AP characteristics distinguish between the phenotypes?

Following the original AP classification by He et al,¹⁵ in human iPSC-CMs, subsequent reports regarding human iPSC-CMs separated APs only into nodal, atrial and ventricular phenotypes^{1,2,16}. In contrast, Wobus et al¹⁷ reported that in mouse embryonic stem cell-derived cardiomyocytes, AP population can be divided into 5 distinct phenotypes, based on the presence/absence of I_f and $I_{K,Ach}$: early pacemaker cells, Pacemaker (AV)-like cells, atrium-like cells, Purkinje-like cells and ventricular-like cells. However, due to the broad phenotype continuum we were unable to classify the AP population into 3 groups using key AP characteristics and objective statistical tools. While it might be suggested that the transitional phenotype we identified could include Purkinje-like cells as described by Wobus et al, the lack of any phase 1 repolarization in the transitional cells argues against a Purkinje phenotype. Moreover, our observation on the phenotype continuity of APs is consistent with the Antzelevitch's group novel findings regarding AP features in human iPSC-CMs: their figure 3 "reveals a nearly continuous range of AP morphologies, highlighting the subjective nature of distinctions made on the basis of AP profiles¹⁸."

The only clearly distinct group was the atrial phenotype easily distinguished by the presence of sharp phase 1. While none of AP characteristics effectively separated the phenotypes, the rate/rhythm features had better discriminatory capability: (1) atrial cells fire faster than the nodal/ventricular cells. (2) BRV is larger in nodal than ventricular cells. While the mechanism underlying this fascinating difference between nodal and ventricular cells was not investigated, it represents a novel feature to discriminate among spontaneously-firing cardiomyocytes of different origins.

The differentiation model

The development-dependent phenotypic profile (Fig. 7A) was generated by: (1) defining the APs with phase 1 spike as atrial; (2) separation of the remaining population into nodal and ventricular, using APD_{90} as an arbitrary discriminating parameter. Despite the inherent limitation of this analysis, it provides a novel description of the development-dependent phenotype distribution. In brief, the nodal phenotype decreases with age in culture, the ventricular phenotype first increases then decreases and subsequently increases again, and the atrial phenotype shows a brief appearance between days 55 and 70. Importantly, these time-dependent changes in the electrophysiological features of the culture are well supported by the FACS results. Hence, in the 2nd group (day 25–56) when the nodal and ventricular APs have similar proportions (Fig. 7), on day 30 (included in this group), there are similar frequencies of nodal/pacemaker and atrial/ventricular markers (Fig. 1). However, as the culture ages, the frequency of the nodal/pacemaker cardiomyocytes decreases (less HCN4-expressing cells) and the atrial/ventricular increases (more MYL2/7-expressing cells), concomitantly with a decrease in the proportion of nodal APs and an increase in the proportion of the atrial/ventricular APs.

In an attempt to explain this complex profile, we propose the following model representing the relative abundance of the different phenotypes (Fig. 7B). A key assumption is that this model reflects a condition whereby the same culture is followed sequentially, rather than the actual circumstance of monitoring different cultures at different time points post-plating.

The situation described in the literature whereby there are only 3 clear AP phenotypes (at any age), can be ‘easily’ modeled; each phenotype originates from its own specific precursor, and the proportion is fixed throughout development in culture (but not necessarily in the embryo). However, due to the following observations this simple model cannot account for the complex developmental-dependent profile presented here: (1) the decrease with time in the nodal proportion; (2) the biphasic ventricular appearance; (3) the transient appearance of the atrial phenotype; (4) the wealth of nodal-to-ventricular transitional phenotypes. The key prerequisite to (begin) explaining these observations is the assumption that the electrophysiological signature of any cell is not fixed, but changes (i.e., interconverts) throughout development. Allowing this flexibility can account for the decline in the nodal phenotype at the expense of the ventricular phenotype, as well as for the broad range of transitional phenotypes which may become either nodal or ventricular, possibly depending on environmental cues such as paracrine effects of non-cardiac cells. Interestingly, as seen in the 3rd age group, the increase in the atrial phenotypes mirrors the marked decrease in the ventricular phenotype. Subsequently, the atrial phenotype disappears and the ventricular phenotype returns. This phenomenon alone may suggest that a certain population expressing a ventricular phenotype can transiently convert into a cardiomyocyte exhibiting an atrial AP. Alternatively, the developmental-dependent changes in the ventricular and atrial abundance are unrelated, and thus result from independent processes, such as differentiation of atrial phenotype from atrial precursors (gray shapes, Fig. 7B). In view of this complex behavior it should be mentioned that the developmental profile as well as the unexpected phenomena of atrial phenotype disappearance could result from the experimental settings, such as: (1) the *in vitro* setting which in contrast to the embryonic heart, lacks the necessary cues that propagate cardiac development in the embryo. (2) The contracting EBs were mechanically dissociated from the original clusters, possibly interrupting important paracrine/mechanical effects. (3) The study included 7–95 day old iPSC-CMs, which is an early developmental period in terms of embryonic life. It is possible that longer culturing would show reappearance of the atrial phenotype.

Conclusions

From the onset of development in culture, the iPSC-CMs population includes the typical nodal, atrial and ventricular AP, but also a broad spectrum of transitional phenotypes. Based on these findings we propose a schematic paradigm to explain the complex nature of iPSC-CMs electrophysiological development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

FACS analysis of cardiac markers on days 30 and 60 day-old iPSC-CMs. [A] 30-day old iPSC-CMs. 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 secondary antibody as control (black dots) or with both troponin T and secondary antibody (gray dots). Troponin⁺ cells were gated according to their relative fluorescence intensity (horizontal axis) and FS properties. [C] Representative and [D] analysis of troponin⁺ cells relative fluorescence intensity of markers for MYL2 (C, blue dots; D, blue bars), MYL7 (C, blue dots; D, blue bars) and HCN4 (C, green dots; D, green bars) as atrial, ventricular and nodal markers, respectively. The frequencies of MYL2/7 and HCN4 were compared at day 30 (C, left panels) and 60 (right panels) days. Each analysis contained 1×10⁶ differentiated cells; four sets of individual experiments were analyzed.

*P<0.001, day 60 MYL2/7⁺ vs. HCN4⁺. For both MYL2/7⁺ and HCN4⁺, #P<0.001, day 30 vs. day 60.

Figure 2.

The different cardiac subpopulations in 30 and 60 day-old iPSC-CMs. [A] Differentiated⁺/Troponin⁺ cells were dot-plotted into subpopulations using antibodies for MYL2 ([A], vertical axis), MYL7 ([A], vertical axis) and HCN4 ([A], horizontal axis). The frequencies of the subpopulation were compared at days 30 ([A], upper panel; [B] black bars) and 60 ([A], lower panel; [B] gray bars). Each analysis contained 1×10^6 differentiated cells; four sets of individual experiments were analyzed. * $P < 0.05$, day 30 vs. day 60 in each of the groups “Low HCN4, MYL2/7⁺”, “HCN4⁺, MYL2/7⁻” and “HCN4⁻, MYL2/7⁺”. ⁺ $P < 0.05$, day 30, group “Hi HCN4, MYL2/7⁺” vs. “Low HCN4, MYL2/7⁺”, “HCN4⁺, MYL2/7⁻” and “HCN4⁻, MYL2/7⁻”. # $P < 0.05$, day 60, group “HCN4⁻, MYL2/7⁺” vs. all other groups and group “HCN4⁻, MYL2/7⁻” vs. groups “Hi HCN4, MYL2/7⁺”, “Low HCN4, MYL2/7⁺” and “HCN4⁺, MYL2/7⁻”.

Figure 3.

The phenotypic distribution of AP recorded from 7- to 95 day old iPSC-CMs and the pacemaker current I_f in young and old iPSC-CMs. [A] Superimposed AP from 7- to 95 day-old iPSC-CMs (n=280). [B] Representative 'typical' ventricular-, nodal- and atrial-like AP shown in panels [E-F] by the red, blue and green traces, respectively. [C-F] AP in the 4 age groups. Cells were analyzed at days: [C] 7-24 (n= 130), [D] 25-56 (n= 54), [E] 57-70 (n=54) and [F] 71-95 (n=42). Green, blue and red traces show atrial, nodal and ventricular AP, respectively, depicted in panel [B]. [G] - [I] I_f in young and old iPSC-CMs. [G] I_f recordings in young (32 days) and old (89 days) iPSC-CMs in the absence (upper panels) and presence (lower panels) of 10 μ M ivabradine. BaCl₂ (500 μ M) was included in the extracellular solution to block I_{K1} . [H] I_f current-voltage relations in young (30-35 days, n=7 cells) and old (63-95 days, n=13) iPSC-CMs. There was a significant statistical difference between the two groups from -80 mV onward. [I] I_f densities at $V_M = -120$ mV. * $P < 0.05$, Young vs. Old iPSC-CMs.

Figure 4.

AP characteristics in 7-to-95 day iPSC-CMs. [A] AP amplitude, overshoot [B], APD₉₀ [C], dV/dt_{max} [D] and MDP [E] (n=203). [F-J] AP characteristics in the 4 age groups (n=37, 44, 70 and 52 respectively). In [F] and [G] *P<0.05, group 71-95 days vs. groups 7-24, 25-56 and 57-70 days. **P<0.05, 57-70 vs. 7-24. In [H] *P<0.05, 71-95 vs. 7-24, 25-56 and 57-70 days. ***P<0.05, 57-70 vs. 25-56. In [I] #P<0.05, 57-70 vs. 7-24, 25-56 and 71-95 days. ##P<0.05, 71-95 vs. 7-24 and 25-56 days.

Figure 5.

The relationships between APD₉₀ and day in culture. [A–D] Histogram distribution and APD₉₀ and best Gaussian fit in the 4 age groups (7–24, 25–56, 57–70, 71–95 days in culture; n=37, 44, 70 and 52 respectively). The Gaussian fit was calculated to automatically identify and quantify populations, using Schwarz criteria. [E] 3D representation of the relationships between dV/dt_{max}, APD₉₀ and iPSC-CM days in culture. Colors indicate the 4 age groups: 7–24 days (blue), 25–56 days (pink), 57–70 days (light green) and 71–95 day (red). See text for details.

Figure 6.

Rate and rhythm characteristics of nodal, ventricular and atrial-like iPSC-CMs. [A–C] representative spontaneous AP recordings from the 3 phenotypes. [D] Combined Inter-Beat Intervals (IBIs) versus time relations in nodal (blue, $n=29$), ventricular (red, $n=44$) and atrial-like (green, $n=36$) iPSC-CMs. [E] Superimposed Poincaré plots of the IBIs. [F] The plot of the Coefficient of Variance of IBI (IBI COV) vs. Beat Rate. The best curve was fitted according to the data. [G] Double-logarithmic plot of IBI COV vs. Beat Rate, showing linear relationship between the two parameters ($P<0.0001$) ($n=109$). [H–K] Summary of Mean Beat Rate [H], COV [I], SD1 [J] and SD2 [K] of the Poincaré plots in ventricular ($n=11$) and nodal-like ($n=8$) iPSC-CMs. The comparisons (using t-test for unpaired observations) in [I–K] included the 2 groups of nodal and ventricular cells firing at similar mean beat rate of 42.7 ± 3.9 and 42.6 ± 3.2 beats/min, respectively. In [I–K] $*P<0.05$. The cells included in the figure were cultured for 57-to-95 days.

Figure 7. Development-dependent changes in action potential phenotype abundance in iPSC-CMs. [A] Frequency distributions of the typical 3 action potential phenotypes and the transitional phenotype, as defined in the text and legend, for the 4 age groups (7–24, 25–56, 57–70, 71–95 days in culture; n=37, 44, 70 and 52 respectively). [B] Proposed schematic model of the phenotypic changes depicted in panel [A]. The colors of the oval shapes in [B] correspond to the colors in [A]. The gray ovals represent atrial precursors. See text for explanation.