Original Paper

Electrophysiological Characteristics of Embryonic Stem Cell-Derived Cardiomyocytes are Cell Line-Dependent

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Key Words

Embryonic stem cells • Cardiomyocytes • Electrophysiology

Abstract

Background: Modelling of cardiac development, physiology and pharmacology by differentiation of embryonic stem cells (ESCs) requires comparability of cardiac differentiation between different ESC lines. To investigate whether the outcome of cardiac differentiation is consistent between different ESC lines, we compared electrophysiological properties of ESC-derived cardiomyocytes (ESC-CMs) of different murine ESC lines. **Methods:** Two wild-type (D3 and R1) and two transgenic ESC lines (D3/aPIG44 and CGR8/AMPIGX-7) were differentiated under identical culture conditions. The transgenic cell lines expressed enhanced green fluorescent protein (eGFP) and puromycin-N-acetyltransferase under control of the cardiac specific α -myosin heavy chain (α MHC) promoter. Action potentials (APs) were recorded using sharp electrodes and multielectrode arrays in beating clusters of ESC-CMs. **Results:** Spontaneous AP frequency and AP duration (APD) as well as maximal upstroke velocity differed markedly between unpurified CMs of the four ESC lines. APD heterogeneity was negligible in D3/aPIG44, moderate in D3 and R1 and extensive in CGR8/AMPIGX-7. Interspike intervals calculated from long-term recordings showed a high degree of variability within and between recordings in CGR8/AMPIGX-7, but not in D3/aPIG44. Purification of the α MHC+ population by puromycin treatment posed only minor changes to APD in D3/aPIG44, but significantly shortened APD in CGR8/AMPIGX-7. **Conclusion:** Electrophysiological properties of ESC-CMs are strongly cell line-dependent and can be influenced by purification of cardiomyocytes by antibiotic selection. Thus, conclusions on cardiac development, physiology and pharmacology derived from single stem cell lines have to be interpreted carefully.

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Introduction

Embryonic stem cells (ESCs) can be differentiated to cardiac myocyte-like cells, so called embryonic stem cell-derived cardiomyocytes (ESC-CMs) [1]. Electrophysiological properties allow distinguishing subtypes among ESC-CMs with properties of sinoatrial, atrial and ventricular cardiomyocytes; the major functional subtypes in the heart [2].

Cardiac differentiation of ESCs recapitulates many aspects of early heart development [3]. The role of signalling cascades that are essential for the specification of mesodermal progenitor cells to the cardiogenic lineage, such as fibroblast growth factor, bone morphogenic protein, Wnt, and Hedgehog pathways, has been amply documented [4]. In addition, transcription factors that control the later cardiac patterning and regionalisation such as *Mesp1*, *NKX2.5*, *TBX5*, *TBX20*, and *GATA4* are essential for cardiac differentiation of ESCs [5]. This makes ESCs a valuable model to study early cardiac development.

Another important application of ESCs is cardiac cell replacement therapy. The regenerative potential of the postnatal heart is insufficient. Therefore, loss of cardiomyocytes, e.g. due to ischemic damage, is virtually irreversible. This could potentially be overcome by transplantation of cardiomyocytes derived from pluripotent stem cells into damaged hearts in order to restore pacemaker [6], conductive [7], or contractile [8] function. Although clinical application of ESC-derived cardiomyocytes (ESC-CMs) is limited by ethical and immunological problems and cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) may overcome these restraints and have successfully been tested in animal studies [9, 10], ESC-CMs remain a widely used and important cell type in experimental cell therapy.

For both studies on cardiac development and cell therapy, a reproducible, stable cardiac differentiation is needed, with comparable properties of ESC-CMs generated from different passages of an individual ESC line, or from different ESC lines. However, previous studies of ESC-CMs have found quite different electrophysiological properties. For instance, a predominantly atrial phenotype of ESC-CMs was found in cells from the D3 line [11], while cells from the GSES line had a higher percentage of ventricular cardiomyocytes [12] and cells from the CGR8 line showed mainly intermediate action potential (AP) properties [13]. Both culture conditions and specific genetic modifications have been shown to influence cardiac subtype differentiation [12, 14]. Since different protocols for ESC culture and differentiation have been applied in those studies, and ESCs were sometimes genetically manipulated to allow identification and purification of cardiomyocytes [11, 13], it remains unclear whether the observed differences in AP properties were caused by inherent differences between ESC lines, or by culture conditions and genetic modifications.

To answer the question of whether different ESC lines possess a comparable potential of cardiac differentiation, or have inherent differences, we compared the electrophysiological parameters of ESC-CMs derived from two wild-type and two transgenic murine ESC lines designated as D3, R1, D3/ α PIG44 (derived from D3) and CGR8/AMPIGX-7 (derived from CGR8) cultured under identical conditions. These cell lines, which have been extensively used in embryonic stem cell research, have a similar genetic background, as D3 cells [15] and R1 cells [16] were established from 129/sv, and CGR8 cells [17] from 1290la mice. The cell lines D3/ α PIG44 [11] and CGR8/AMPIGX-7 [13] stably express enhanced green fluorescent protein (eGFP) and a puromycin resistance cassette under control of the α -myosin heavy chain (α MHC) promoter, allowing purification of cardiomyocytes by addition of puromycin.

Materials and Methods

Culture and differentiation of ES cells

The two transgenic ES cell lines used in this study were genetically modified with a construct carrying a puromycin-acetyltransferase and eGFP under the control of the α MHC promoter as reported previously, and are designated as D3/ α PIG44 derived from the ESC line D3 [11] and CGR8/AMPIGX-7 derived from the ES cell line CGR8 [13]. In addition, we used wild-type D3 [15] and R1 [16] cell lines.

All four cell lines were treated using an identical cell culture protocol. ESCs were maintained on feeder cells in Iscove's modified Dulbeccos medium (IMDM), supplemented with 17% fetal calf serum (FCS), 100 U/ml penicillin, 100 µg/ml streptomycin, 1% non-essential amino acids (1%), 0.1 mM 2-mercaptoethanol (all purchased from Invitrogen, Karlsruhe, Germany) and 1000 IU/ml Leukemia Inhibiting Factor (ESGRO, Millipore, Billerica, MA, USA). Every other day, ESCs were washed with D-PBS, trypsinised, centrifuged, and diluted to 500,000 cells/dish.

Differentiation was induced by the formation of hanging drops. Briefly, dissociated ESCs were diluted to 20,000 cells/ml. Drops of 20 µl were formed on the top lid of the culture dishes. Formation of embryoid bodies (EBs) occurred within two days. After two days, EBs were transferred to the suspension culture. For purification of CGR8/AMPIGX-7 and D3/αPIG44, fluorescence was checked on day 9 and puromycin (7.5 µg/ml) was added. On day 12, medium was changed and puromycin was added a second time.

AP recordings

Electrophysiological measurements were performed on day 14 of differentiation. APs were recorded in spontaneously beating areas of EBs or in beating clusters after purification. Field stimulation with two titanium bath electrodes equidistant to the recording electrodes (biphasic pulses with a pulse amplitude and duration 50% above threshold of the spontaneously beating ESC-CMs) was used to identify quiescent ESC-CMs. To avoid a bias towards eGFP⁺ cells, fluorescence was not observed during AP recordings.

Temperature was kept at 37°C and EBs were superfused with DMEM (high glucose, HEPES buffered; Invitrogen, Karlsruhe, Germany). Oxygen tension and pH were controlled by carbogen bubbling. APs were recorded with microelectrodes pulled of borosilicate capillaries (WPI, Sarasota, USA) filled with 3 M KCl solution at resistances between 30-60 MΩ. The signal was amplified by a SEC-10LX (npi electronics, Tamm, Germany) and digitised with a HEKA EPC-9 controlled by the Pulse software (HEKA Systems, Lambrecht/Pfalz, Germany). APs were analysed using the MiniAnalysis software (Synaptosoft, Fort Lee, NJ, USA).

Field potential recordings

Multielectrode arrays (MEAs, Multi Channel Systems, Reutlingen, Germany) were plasma-treated and coated with gelatine (0.1% in D-PBS) for at least two hours at 37°C. The MEAs used in this study had 60 TiN electrodes with diameters of 30 µm and interelectrode distances of 200 µm arranged in 8 rows and 8 columns. Beating EBs were selected on day 12 of differentiation, incubated with IMDM supplemented with 20% FCS and allowed to attach to the MEA. After two days, electrical activity was measured using a MEA1060 amplifier (Multi Channel Systems, Reutlingen, Germany) for 10 min. Temperature was kept at 37°C. Data were digitised with a sampling frequency of 5 kHz and recorded with the MCRack software (Multi Channel Systems, Reutlingen, Germany). Interspike intervals between recorded field potentials were analysed off-line using custom-written routines in Matlab 5.3 (The Mathworks, Natick, MA, USA).

Statistical analysis

Data were statistically analysed using IBM SPSS Statistics 22 (IBM, Armonk, NY, USA). If not indicated otherwise, values are given as mean ± standard error of the mean (SEM).

Independent samples were compared using one-way ANOVA. For paired samples, a paired-samples t-test was applied. Statistical significance was assumed at $p < 0.05$.

Results

AP properties of unpurified ESC-CMs

AP waveforms and frequencies were markedly different between ESC-CMs derived from the four different cell lines CGR8/AMPIGX-7 (Fig. 1A,B), D3/αPIG44 (Fig. 1C,D), D3 (Fig. 1E,F), and R1 (Fig. 1G,H). AP frequency was significantly higher in unpurified D3/αPIG44 ESC-CMs than in the three other cell lines (Fig. 1I, D3/αPIG44: 7.3 ± 0.2 Hz, $n = 62$, CGR8/AMPIGX-7: 1.7 ± 0.2 Hz, $n = 20$, D3: 2.7 ± 0.1 , $n = 13$, R1: 2.1 ± 0.2 , $n = 12$, all $p < 0.001$). Maximum upstroke velocity ($V_{\max}$) was higher in D3/αPIG44 compared to CGR8/AMPIGX-7 (Fig. 1J, D3/αPIG44: 37.1 ± 2.2 V/s, $n = 62$ vs CGR8/AMPIGX-7: 22.1 ± 2.0 V/s, $n = 20$, $p <$

Fig. 1. Representative AP waveforms, spontaneous AP frequency and upstroke velocity of unpurified ESC-CMs. (A-H) Representative APs of unpurified ESC-CM derived from lines CGR8/AMPIGX-7 (A, B), D3 (C, D), D3/αPIG44 (E, F) and R1 (G, H). (I) Differences in spontaneous AP frequency between cell lines. (J) Maximal upstroke velocity (V_{max}) varied between cell lines. While V_{max} was higher in R1 compared to wild-type D3 and D3/αPIG44, it was lower in CGR8/AMPIGX-7. *** $p < 0.001$ vs. D3/αPIG44.

Fig. 2. Cell-line dependent differences in action potential duration (APD) between unpurified ESC-CMs. Each data point represents one recording. APD20, APD50 and APD90 were considerably longer in CGR8/AMPIGX-7 than in D3/αPIG44. CGR8 showed the highest dispersion of APD compared to the other three cell lines. APD20, APD50 and APD90 were also significantly longer in D3 and R1 than in D3/αPIG44.

0.001), while it was lower compared to R1 ($R1: 57.4 \pm 7.8$ V/s, $n = 12$, $p = 0.001$) and similar to D3 ($D3: 34.6 \pm 5.3$ V/s, $n = 13$, $p = 0.64$). Within all cell lines, we observed a considerable cell-to-cell dispersion of V_{max} reflected by a high range (D3/αPIG44 10.3 – 93.1 V/s, CGR8/AMPIGX-7 9.6 – 49.5 V/s, D3 5.1 – 58.7 V/s, R1 9.8 – 112.0 V/s) and a high coefficient of variation (D3/αPIG44 46.8%, CGR8/AMPIGX-7 41.5%, D3 55.3%, R1 47.6%).

CGR8/AMPIGX-7 ESC-CMs had markedly longer APs than D3/αPIG44 ESC-CMs (Fig. 2) (CGR8/AMPIGX-7: $n = 20$, APD20: 36.0 ± 5.0 ms, APD50: 80.6 ± 10.2 ms, APD90: 188.9 ± 13.2 ms vs D3/αPIG44: $n = 62$, APD20: 7.0 ± 0.2 ms, APD50: 14.5 ± 0.4 ms, APD90: 28.9 ± 0.5 ms, all $p < 0.001$). APD was also longer in D3 ($n = 13$, APD20 15.9 ± 0.7 ms, APD50 31.9 ± 1.2 ms, APD90 58.7 ± 2.5 ms) and R1 ($n = 12$, APD20 12.5 ± 0.5 ms, APD50 28.2 ± 1.2 ms, APD90 56.9 ± 1.6 ms) (Fig. 2). However, ratios between APD20 and APD50, as well as between APD50 and APD90, were cell line-independent. Both showed a strong linear correlation (Spearman-Rho, $R = 0.938$ for APD20 and APD50, $R = 0.927$ for APD50 and APD90, $p < 0.001$ in both cases, $n = 107$).

Fig. 3. Effect of purification on APD. Each data point represents one recording. (A) We observed a slight prolongation of APD20 and APD50 in purified D3/ α PIG44 compared to unpurified D3/ α PIG44. A cut-off APD that distinguishes purified and unpurified D3/ α PIG44 could not be determined. APD90 was not significantly different between purified and unpurified D3/ α PIG44. (B) Purified CGR8/AMPIGX-7 showed a shorter mean APD50 and APD90 compared to unpurified CGR8/AMPIGX-7. This was due to a loss of ESC-CMs with very long APD.

Effects of purification on AP parameters

While AP frequency and APD90 were not significantly different between unpurified and purified D3/ α PIG44 ESC-CMs (AP frequency: 7.3 ± 0.2 Hz, $n = 62$ vs. 6.9 ± 0.1 Hz, $n = 48$, $p = 0.177$, APD90 28.9 ± 0.5 ms, $n = 62$ vs. 29.8 ± 0.3 ms, $n = 48$, $p = 0.122$), APD20 and APD50 were slightly prolonged in purified D3/ α PIG44 ESC-CMs (APD20: 7.0 ± 0.2 ms, $n = 62$ vs. 8.3 ± 0.3 ms, $n = 48$, $p = 0.004$, APD50: 14.5 ± 0.4 ms, $n = 62$ vs. 16.1 ± 0.3 ms, $n = 48$, $p = 0.001$) (Fig. 3A). $V_{\max}$ was increased in purified D3/ α PIG44 ESC-CMs (37.1 ± 2.2 V/s, $n = 62$ vs. 66.3 ± 5.2 V/s, $n = 48$, $p < 0.001$).

In contrast, APD50 and APD90 were shortened in purified CGR8/AMPIGX-7 (APD50: 80.6 ± 10.2 ms, $n = 20$ vs. 50.1 ± 6.1 ms, $n = 12$, $p < 0.05$, APD90: 188.9 ± 13.2 ms, $n = 20$ vs. 79.8 ± 7.2 ms, $n = 12$, $p < 0.05$) mainly due to a loss of CGR8/AMPIGX-7 ESC-CMs with very long AP durations (Fig. 3B). APD20, $V_{\max}$ and AP frequency were not significantly different.

Variability of interspike intervals (ISIs)

To assess spontaneous beating rate and its variability, we recorded the electrical activity of unpurified D3/ α PIG44 and CGR8/AMPIGX-7 ESC-CMs with MEAs over 10 minutes. Representative tachograms are given in Fig. 4A (D3/ α PIG44) and Fig. 4B (CGR8/AMPIGX-7). Consistent with the intracellular recordings, we found shorter ISIs in D3/ α PIG44 ESC-CMs compared to CGR8/AMPIGX-7 ESC-CMs after 12+2 days of differentiation (Fig. 4C, D3/ α PIG44: 250.0 ± 19.7 ms, range 210.2 ms – 324.0 ms, $n = 32$ vs. CGR8/AMPIGX-7: 1147.0 ± 85.6 ms, $n = 50$, range 556.7 ms – 2305.0 ms, $p < 0.001$).

As mean ISIs were more than four times longer in CGR8/AMPIGX-7 than in D3/ α PIG44 ESC-CMs, we used the coefficient of variation as a measure of variability as it is normalised to the mean. We calculated the coefficient of variation within each individual measurement. CGR8/AMPIGX-7 showed a higher coefficient of variation compared to D3/ α PIG44 (Fig. 4D, D3/ α PIG44: $5.9 \pm 1.4\%$, $n = 32$ vs. CGR8/AMPIGX-7: $21.1 \pm 2.2\%$, $n = 51$, $p < 0.001$).

Effects of cardioactive drugs

Both unpurified D3/ α PIG44 and CGR8/AMPIGX-7 were sensitive to the beta-adrenergic agonist isoproterenol and the subsequently applied muscarinic agonist carbachol (Fig. 5A,B). After isoproterenol application ($1 \mu\text{M}$), mean ISI decreased by 33.8% in D3/ α PIG44

Fig. 4. Variability of interspike intervals (ISIs). (A) Tachogram (MEA recording, D3/αPIG44, 10 min). D3/αPIG44 showed a regular beating with relatively short ISIs. (B) Tachogram (MEA recording, CGR8/AMPIGX-7, 10 min). CGR8/AMPIGX-7 showed longer ISIs and a high variability of ISI duration. (C) Mean ISI duration. Mean ISIs were shorter in D3/αPIG44 (ISI duration: 250 ± 19.7 ms, range 210.2 ms – 324.0 ms, $n = 32$) than in CGR8/AMPIGX-7 (ISI duration: 1147 ± 85.6 ms, range 556.7 ms – 2305.0 ms, $n = 50$, $p < 0.001$). (D) Dispersion of ISI duration within individual measurements. Irregular beating in CGR8/AMPIGX-7 corresponds to a higher coefficient of variation than in D3/αPIG44.

(before isoproterenol: 253.5 ± 24.4 ms, with isoproterenol: 167.9 ± 15.6 ms, $n = 9$, $p < 0.001$) and by 47.8% in CGR8/AMPIGX-7 (before isoproterenol: 1444.0 ± 193.3 ms, with isoproterenol: 752.6 ± 109.8 ms, $n = 13$, $p < 0.001$). After carbachol application (10 μ M), mean ISI increased by 45.0 % in D3/αPIG44 (after carbachol: 305.2 ± 20.3 ms, $n = 9$, $p < 0.01$) and by 43.4 % in CGR8/AMPIGX-7 (after carbachol: 1329.3 ± 373.5 ms, $n = 13$, $p < 0.01$).

CsCl (4 mM), a blocker of the hyperpolarisation-activated current I_h , increased ISI in D3/αPIG44 by 15.4% (Fig. 5 C,D) (before CsCl: 296.3 ± 69.6 ms, with CsCl 350.2 ± 56.6 ms, $n = 7$, $p < 0.001$). In CGR8/AMPIGX-7, ISI duration was not affected by CsCl (before CsCl: 1364.3 ± 209.8 ms, with CsCl: 1325.7 ± 236.7 ms, $n = 10$, not significant). Nifedipine (1 μ M), a blocker of the voltage activated L-type calcium channel, led to a complete, but reversible cessation of beating in both D3/αPIG44 and CGR8/AMPIGX-7 (Fig. 5E,F).

Discussion

In the present study, we compared the electrophysiological characteristics of CMs derived from different murine ESC lines under identical culture conditions. We found (i) a substantial cell line-dependent variation of AP frequency, maximal upstroke velocity and APD, (ii) a cell line-dependent effect of CM purification by antibiotic selection, (iii) a substantial cell line-dependent difference in beating regularity, and (iv) differences in the response to the I_h -blocker CsCl.

Fig. 5. Effects of cardioactive drugs. (A,B) D3/ α PIG44 and CGR8/AMPIGX-7 responded to adrenergic (isoproterenol 1 μ M) and muscarinic (carbachol 10 μ M) stimulation. In D3/ α PIG44, mean ISI decreased from 253.5 ± 24.4 ms to 167.9 ± 15.6 ms with isoproterenol ($n = 9$, $p < 0.001$). After application of carbachol, mean ISI increased to 305.2 ± 20.3 ms ($n = 9$, $p < 0.01$). In CGR8/AMPIGX-7, mean ISI decreased from 1444.0 ± 193.3 ms to 752.6 ± 109.8 ms with isoproterenol ($n = 13$, $p < 0.001$). After application of carbachol, mean ISI increased to 1329.3 ± 373.5 ms ($n = 13$). (C,D) Inhibition of I_f by CsCl. ISI decreased from 296.3 ± 69.6 ms to 350.2 ± 56.6 ms with application of CsCl (4 mM) in D3/ α PIG44 ($n = 7$, $p < 0.001$). In CGR8/AMPIGX-7, ISI duration was not affected by CsCl (Baseline: 1364.3 ± 209.8 ms vs. CsCl 5 mM: 1325.7 ± 236.7 ms, $n = 10$). (E,F) Inhibition of L-type calcium channels by nifedipine (1 μ M) led to complete, but reversible cessation of beating.

It has previously been observed that a substantial variation in the differentiation efficiency exists between different ESC lines. Pekkanen-Mattila et al. compared the cardiac differentiation of eight human ESC lines derived and cultured under the same conditions [18]. Differentiation efficiency ranged from 0% to ~9%. A systematic comparison of five different human ESC lines [19] showed that embryoid body number and size, as well as the expression of germ layer markers, vary among the different ESC lines. It was therefore suggested that the part of the inner cell mass that contributes to the cell line could already be committed to a confined phenotype [18].

In addition to the known differences in differentiation efficiency, the present study shows that the variation of cardiac differentiation extends to the electrophysiological characteristics of ESC-CMs. Previous studies have demonstrated that ESC can give rise to cardiomyocytes with different properties, including sinoatrial, atrial and ventricular cardiomyocytes [2]. Subtype differentiation has been reported to be influenced by culture conditions and genetic modifications, which enable the purification of subsets of cardiomyocytes or precursor cells expressing certain factors [12, 14]. Studies involving ESC-CM of different origin have shown electrophysiological differences between studied cells [11, 12, 13]. However, it was unclear

so far, whether this was caused by inherent functional differences of ESC lines or by varying culture conditions and genetic modifications. Our direct comparison of electrophysiological properties of different ESC lines, cultured and differentiated according to the same protocol, demonstrates that inherent differences of different ESC lines are indeed present. This finding raises important issues: Firstly, cardiac differentiation of ESCs as a model of cardiac development has to be interpreted with caution. However, the comparison of functional data of different ESC lines might give insight into the development of specific electrophysiological characteristics. Secondly, if pluripotent stem cell-derived CMs do come to clinical use, it will be necessary to select the optimal cell line and differentiation protocol that meet the specific functional properties that cells are supposed to restore.

In the literature, ESC-CMs are generally classified as sino-atrial, atrial and ventricular cardiomyocytes based on AP morphology [2]. Therefore, one might ask whether the distribution of cardiomyocyte subtypes depends on the cell line. However, given the data presented in our study, we refrained from classifying ESC-CMs into the different subtypes, because the spectrum of APDs did not provide cut-off values that would enable unequivocal allocation of APs to different functional subtypes. In addition, we were not able to allocate functional subtypes based on AP morphology only, as we often observed intermediate AP morphologies, which did not show unambiguous features of specific cell types. A potential explanation for this observation could be that, in contrast to reports on different electrophysiological subtypes in ESC-CMs [2], we did not use dissociated cells, but ESC-CMs within clusters with preserved electrical coupling.

Measurements were performed after 14 days of differentiation, as we wanted to avoid prolonged culture times as a potential confounding factor. In addition, a high degree of purity can be achieved after antibiotic selection of D3/aPIG44 and CGR8/AMPIGX-7 ESC-CMs at this time [11, 13]. Day 14 post coitum has also been used frequently for experiments with murine fetal cardiomyocytes.

Electrophysiological differences have been reported previously between iPSC-derived CMs and ESC-CMs, and it has been suggested that this might be an effect of epigenetic changes during reprogramming or incomplete reprogramming [20]. Sheng et al. reported that sodium current density and maximal upstroke velocity were lower in CMs derived from the ESC line HES2 than in CMs derived from the iPSC line C1, while calcium current density was not different [21]. In murine cell lines, sodium current density was lower in iPS-CMs than in ESC-CMs, while calcium current density was higher [20]. In the present study, we observed that considerable differences also exist between different ESC lines, and it therefore seems unlikely that the differences between iPSC and ESC lines are exclusively a side effect of reprogramming.

We can only speculate on the differences in ion channel expression underlying the observed cell-line dependent electrophysiological differences. All major cardiac ionic currents including the 'funny' current I_f , L-type and T-type calcium currents, voltage-activated sodium current and various potassium currents ($I_{K'}$, I_{to} , I_{K1} , $I_{K,Ach}$, $I_{K,ATP}$) have been reported in ESC-CMs [20-26]. In the present study, we observed major differences in the rate of automaticity, maximal upstroke velocity and APD. In ESC-CMs, automaticity seems to depend on the interplay between L-type [27] and T-type [26] calcium currents, I_f [23, 24, 26] and intracellular Ca^{2+} -oscillations [28]. As an indication that a different expression of I_f might partly be responsible for the different degrees of automaticity, we observed no response to the I_f blocker CsCl in CGR8/AMPIGX-7, but a reduction of AP frequency in D3/AMPIGX-7. The considerable difference in APD between cell lines suggests a different potassium channel expression, as the respective currents are predominantly responsible for AP repolarization.

In summary, we could show a substantial cell line-dependent variation of electrophysiological properties of ESC-CMs, demonstrating that ESC lines possess inherent functional differences. This finding has to be taken into consideration, if ESC-CMs are used in developmental and pharmacological studies or for therapeutical approaches.

Abbreviations

α MHC (α -myosin heavy chain); AP (Action potential); APD (Action potential duration); DMEM (Dulbecco's modified Eagle's medium); EB (Embryoid body); eGFP (Enhanced green fluorescent protein); ESC (Embryonic stem cell); ESC-CM (Embryonic stem cell-derived cardiomyocyte); iPSC (Induced pluripotent stem cell); ISI (Interspike interval); PSC (Pluripotent stem cell); TdP (Torsade de pointes); $V_{\max}$ (Maximal upstroke velocity).

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